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Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Appendix 2

Content

Supplementary Methods

Enzyme-linked immunosorbent assay for IgG and IgA (ELISA)

According to the manuals of the RBD-binding IgG ELISA kit (Beijing Kewei) or IgA ELISA kit (Beijing Kewei), heat-inactivated human serum samples at a 1:40 dilution and the diluted reference standard were added in duplicate to the rSARS-CoV-2 RBD-precoated wells and incubated for 30 min at 37°C. The microplates were washed, and then horseradish peroxidase (HRP)-conjugated goat anti-human IgG or IgA antibody was added to bind with the human antibody bound to the rSARS-CoV-2 RBD protein for 30 min. The microplates were read after a colorimetric signal was generated by the addition of TMB chromogenic substrate for 15 min. The total anti-rSARS-CoV-2 RBD IgG or IgA antibody levels in a serum sample were quantitated in ELISA units (EU)/ml by comparison to a reference standard curve. The results were analysed by GraphPad Prism 8.0 using 4-PL curve fitting. IgA standard curves were created from reference serum from a pool of high-titer convalescent patients, and IgG standard curves were created from monoclonal antibodies against SARS-CoV-2 RBD.

IgG subclass ELISA

SARS-CoV-2-RBD-specific IgG1, IgG2, IgG3, and IgG4 responses were detected by optical density (OD) ELISA. Briefly, ELISA plates coated with recombinant SARS-CoV-2 spike RBD protein in this assay were obtained from the RBD-binding IgG ELISA kit (Beijing Kewei Inc.). Heat-inactivated human serum samples at a 1:40 dilution were added in duplicate to the rSARS-CoV-2 RBD-precoated wells and incubated for 30 min at 37°C. Biotinylated anti-human IgG1, anti-human IgG2, anti-human IgG3, and anti-human IgG4 (Mabtech) at a 1:2000 dilution were added to the plates, incubated for 30 min, and then incubated for 30 min with streptavidin-HRP (Mabtech). Finally, TMB chromogenic substrate was used for colour development, and the microplates were read.

SARS-CoV-2 Plaque Reduction Neutralisation Test ($PRNT_{50}$).

A 50% plaque reduction neutralisation test (PRNT50) was used for the SARS-CoV-2 neutralisation assay. The heat-inactivated human serum samples were diluted in duplicate to an initial dilution of 1:8 followed by a 3-fold dilution series. The virus (50 µl, 2000 plaque-forming units [PFU]/ml) was mixed with an equal volume of each diluted serum sample and incubated for 1 hour. Then, the mixture was transferred to a Vero E6 cell monolayer on 24-well plates for 1 hour of incubation. Then, the virus-serum mixture was removed, and 0.5 ml/well culture medium with 0.9% methylcellulose was added to the plates. The plates were returned to 37°C for 4-5 days. Cells were stained with 0.5% crystal violet solution, and the plaques were counted using a Celigo Imaging Cytometer (Nexcelom Bioscience, USA). Neutralisation results were analysed by the Reed-Muench method to estimate the dilution of sera required for half-maximal neutralisation of infection. The initial dilution of sera (1:8) was set as the limit of confidence of the assay.

Ad5 neutralisation assay

The Ad5 neutralisation assay was based on the firefly luciferase assay system. The heat-inactivated human serum samples were diluted in duplicate to an initial dilution of 1:12 and followed by a 3-fold dilution series. No serum was added to the positive control wells, which resulted in the maximum luciferase activity for calculating the 90% neutralisation values. The Ad5-Luciferase was mixed with an equal volume of each diluted serum sample and incubated for 1 hour in the 96-well plate. Then A549 cell suspension was added to the mixture. After 24 hours of culture in 37°C, Cells were washed and lysed. The Luciferase activity was determined using Firefly Luciferase Assay system (Promega) and the value was read out using GloMax Microplate luminometer (Promega).

Intracellular cytokine staining

Freshly isolated PBMCs were restimulated for 6 hours at 37°C with or without a SARS-CoV-2 spike protein peptide pool (1.0 µg ml^{-1}/peptide; GL Biochem (Shanghai) Ltd.) in the presence of 1.0 µg ml^{-1} of anti-human CD28 and CD49d (BioLegend), GolgiStop (0.67 µl ml^{-1}) and GolgiPlug (1 µl ml^{-1}) (BD Biosciences). The peptides were 15-mers overlapping by 11 amino acids and covered the full length of the SARS-CoV-2 spike protein sequence. Cells were washed once with PBS and stained with the viability dye Near-IR for 15 min in the dark at room temperature. After one wash with PBS, a cocktail of surface antibodies containing anti-human CD3 PerCP/Cy5.5 (1:100), CD4 AF700 (1:100), CD8 BV510 (1:50), CD14 APC/Cy7 (1:100), CD19 APC/Cy7 (1:100), CD45RA FITC (1:50) and CCR7 PE/Cy7 (1:12.5) (BioLegend) was added and incubated in the dark at room temperature for 20 min. Afterwards, samples were fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) according

to the manufacturer's instructions. The intracellular cytokines were stained in Perm/Wash buffer (BD Biosciences) in the dark for 30 min at room temperature (IFNγ AF647, 1:50; IL-2 BV421, 1:25; IL-4 BV605, 1:50; IL-13 PE, 1:25; all BioLegend). Samples were washed successively with Perm/Wash buffer and PBS and resuspended in PBS. Data were acquired on a FACS Canto™ instrument (BD Biosciences) and analysed by FACS Diva software. A hierarchical gating strategy was applied for sample analysis (Figure S7). SARS-CoV-2 spike protein-specific responses were calculated by subtraction of the unstimulated controls from the peptide-stimulated samples, and negative values were set to zero.

IFNγ enzyme-linked immunospot (ELISPOT) assay

ELISpot assays were performed on fresh PBMCs by a human IFNγ ELISpot Kit (Mabtech) following the manufacturer's instructions. Tests were performed in triplicate and with positive controls in duplicate (100 ng ml^{-1} phorbol 12-myristate 13-acetate and 1.0 μg ml^{-1} ionomycin (Sigma)). The precoated ELISpot plates were washed with sterile PBS and blocked with RPMI 1640 medium containing 10% foetal bovine serum and 1 × penicillin-streptomycin solution (Gibco) for at least 30 min at room temperature. Then, 1×10^5 PBMCs were added to each test well of the plate along with the SARS-CoV-2 spike protein peptide pool (1 μg ml^{-1}/peptide) or the same volume of DMSO for unstimulated controls, and 1×10^4 PBMCs were added to each positive control well. The cells were incubated for 16-24 hours in a 37°C humidified incubator with 5% CO_2. On the next day, the plates were washed 5 times with PBS, incubated for 2 hours at room temperature with 1 μg ml^{-1} of the detection antibody (7-B6-1-biotin), washed, incubated for 1 hour at room temperature with streptavidin-HRP (1:1000), washed, and developed spots with the TMB substrate. The plates were washed extensively with deionized water to stop spot development, dried in the dark, and then counted using a CTL automated ELISpot counter (Cellular Technology Limited). The counts were summarized as the mean values of triplicate wells with the values of the unstimulated wells subtracted, and negative values were set to zero. The results are expressed as SARS-CoV-2 spike protein-specific spot-forming cells (SFCs) per 10^5 PBMCs. Responses were considered positive if there were ≥5 spike protein-specific SFCs per 10^5 PBMCs and the ratio of spots in stimulated wells to background wells was no less than 2.1.

Cytokine profiling

Freshly isolated PBMCs were restimulated for 24 hours with a SARS-CoV-2 spike protein peptide pool (1 μg ml^{-1}/peptide) or stimulated with an equal volume of DMSO as a negative control. Supernatants were collected to detect the concentrations of IFNγ, TNF-α, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F and IL-22 using a bead-based 12-plex human Th cytokine multianalyte flow assay kit (BioLegend) according to the manufacturer's instructions. Data were acquired on a FACS Canto™ (BD Biosciences) flow cytometer and analysed by LEGENDplex™ data analysis software (BioLegend). Spike protein-specific cytokine production was corrected by subtracting the values obtained from negative controls. Negative values were set to zero.

Supplementary results

Aerosol Ad5-nCoV and vaccination

The nebulizer for the aerosol administration (Aerogen, USA) is a commercial product and has shown stable nebulization performance. After nebulization, more than 70% of aerosols have a particle size of less than 5.4 μm. It has been reported that aerosol particles in the range of 1 to 5 μm will allow a high deposition in the lungs. The residual vaccine remaining in the nebulizer is 4.2% of the total amount of vaccine loaded (95% CI, 2.1%-6.2%). 81.8% (95% CI, 79.2%-84.4%) of the vaccine can be recovered from the aerosol generated by the nebulizer. A breathing simulator or a respirator was used to simulate the inhalation process to study the administration efficiency, and 43.3% (95% CI, 35.9%-50.8%) of the total dose was inhaled into the breathing simulation system.

Before vaccination, nurses trained all participants under the standard operating procedures of aerosol vaccination to reduce differences in personal breathing methods.

Supplementary tables and figures

Table S1. Percentage of participants experiencing solicited adverse events by symptom, vaccination dose, vaccine group, and maximum NMPA toxicity grade*

Symptom	Vaccination	Group	N	Maximum Severity（%）				Total (%)
				Grade 1	Grade 2	Grade 3	Grade 4	
Any	1	HDmu	26	15.4	7.7	0	0	23.1
		LDmu	26	15.4	7.7	3.8	0	26.9
		MIX	26	34.6	15.4	7.7	0	57.7
		1Dim	26	38.5	26.9	7.7	0	73.1
		2Dim	26	30.8	19.2	7.7	0	57.7
	2	HDmu	26	23.1	34.6	11.5	0	69.2
		LDmu	26	34.6	15.4	15.4	0	65.4
		MIX	25	36.0	12.0	4.0	0	52.0
Fever	1	HDmu	26	7.7	0	0	0	7.7
		LDmu	26	3.8	3.8	3.8	0	11.4
		MIX	26	19.2	11.5	7.7	0	38.4
		1Dim	26	7.7	23.1	7.7	0	38.5
		2Dim	26	23.1	7.7	7.7	0	38.5
	2	HDmu	26	23.1	26.9	11.5	0	61.5
		LDmu	26	15.4	11.5	15.4	0	42.3
		MIX	25	20.0	8.0	4.0	0	32.0
Fatigue	1	HDmu	26	7.7	0	0	0	7.7
		LDmu	26	7.7	0	0	0	7.7
		MIX	26	11.5	3.8	0	0	15.3
		1Dim	26	23.1	0	0	0	23.1
		2Dim	26	11.5	0	0	0	11.5
	2	HDmu	26	42.3	0	0	0	42.3
		LDmu	26	38.5	7.7	0	0	46.2
		MIX	25	16.0	0	0	0	16.0
Headache	1	HDmu	26	3.8	3.8	0	0	7.6
		LDmu	26	7.7	0	0	0	7.7
		MIX	26	15.4	0	0	0	15.4
		1Dim	26	11.5	7.7	0	0	19.2
		2Dim	26	19.2	7.7	0	0	26.9
	2	HDmu	26	23.1	23.1	0	0	46.2
		LDmu	26	30.8	11.5	0	0	42.3
		MIX	25	24.0	0	0	0	24.0
Myalgia	1	HDmu	26	0	0	0	0	0
		LDmu	26	11.5	0	0	0	11.5
		MIX	26	0	0	0	0	0
		1Dim	26	11.5	0	0	0	11.5
		2Dim	26	7.7	0	0	0	7.7
	2	HDmu	26	53.8	0	0	0	53.8
		LDmu	26	23.1	3.8	0	0	26.9
		MIX	25	4.0	0	0	0	4.0
Arthralgia	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	0	0	0	0	0
		1Dim	26	19.2	0	0	0	19.2
		2Dim	26	3.8	0	0	0	3.8
	2	HDmu	26	11.5	0	0	0	11.5
		LDmu	26	7.7	0	0	0	7.7
		MIX	25	0	0	0	0	0
Nausea	1	HDmu	26	0	0	0	0	0
		LDmu	26	7.7	0	0	0	7.7
		MIX	26	3.8	0	0	0	3.8
		1Dim	26	3.8	0	0	0	3.8
		2Dim	26	7.7	0	0	0	7.7
	2	HDmu	26	15.4	0	0	0	15.4
		LDmu	26	3.8	0	0	0	3.8
		MIX	25	4.0	0	0	0	4.0
Fainting	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	0	0	0	0	0
		1Dim	26	0	0	0	0	0
		2Dim	26	0	0	0	0	0

	2	HDmu	26	0	0	0	0	0
		LDmu	26	3.8	0	0	0	3.8
		MIX	25	0	0	0	0	0
Cough	1	HDmu	26	0	0	0	0	0
		LDmu	26	7.7	0	0	0	7.7
		MIX	26	0	0	0	0	0
		1Dim	26	7.7	0	0	0	7.7
		2Dim	26	0	0	0	0	0
	2	HDmu	26	11.5	0	0	0	11.5
		LDmu	26	0	0	0	0	0
		MIX	25	8.0	4.0	0	0	12.0
Dyspnoea	1	HDmu	26	3.8	0	0	0	3.8
		LDmu	26	0	0	0	0	0
		MIX	26	0	0	0	0	0
		1Dim	26	0	0	0	0	0
		2Dim	26	0	0	0	0	0
	2	HDmu	26	11.5	0	0	0	11.5
		LDmu	26	0	0	0	0	0
		MIX	25	0	0	0	0	0
Diarrhe	1	HDmu	26	3.8	0	0	0	3.8
		LDmu	26	0	0	0	0	0
		MIX	26	0	0	0	0	0
		1Dim	26	0	0	0	0	0
		2Dim	26	0	0	0	0	0
	2	HDmu	26	0	0	0	0	0
		LDmu	26	3.8	0	0	0	3.8
		MIX	25	0	0	0	0	0
Appetite impaired	1	HDmu	26	0	0	0	0	0
		LDmu	26	7.7	0	0	0	7.7
		MIX	26	0	0	0	0	0
		1Dim	26	3.8	0	0	0	3.8
		2Dim	26	3.8	0	0	0	3.8
	2	HDmu	26	11.5	0	0	0	11.5
		LDmu	26	3.8	3.8	0	0	7.6
		MIX	25	4.0	0	0	0	4.0
Pain at injection site	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	26.9	0	0	0	26.9
		1Dim	26	53.8	0	0	0	53.8
		2Dim	26	34.6	7.7	0	0	42.3
	2	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	25	0	0	0	0	0
Redness at injection site	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	7.7	0	0	0	7.7
		1Dim	26	7.7	0	0	0	7.7
		2Dim	26	3.8	0	0	0	3.8
	2	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	25	0	0	0	0	0
Swelling at injection site	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	11.5	0	0	0	11.5
		1Dim	26	7.7	0	0	0	7.7
		2Dim	26	3.8	0	0	0	3.8
	2	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	25	0	0	0	0	0
Induration at injection site	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	3.8	0	0	0	3.8
		1Dim	26	3.8	0	0	0	3.8
		2Dim	26	3.8	0	0	0	3.8
	2	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	25	0	0	0	0	0
Dry mouth	1	HDmu	26	0	0	0	0	0
		LDmu	26	3.8	0	0	0	3.8
		MIX	26	0	0	0	0	0
		1Dim	26	3.8	0	0	0	3.8

		2Dim	26	0	0	0	0	0
	2	HDmu	26	11.5	0	0	0	11.5
		LDmu	26	3.8	0	0	0	3.8
		MIX	25	12.0	0	0	0	12.0
Oral mucositis	1	HDmu	26	3.8	0	0	0	3.8
		LDmu	26	3.8	0	0	0	3.8
		MIX	26	0	0	0	0	0
		1Dim	26	0	0	0	0	0
		2Dim	26	0	0	0	0	0
	2	HDmu	26	3.8	0	0	0	3.8
		LDmu	26	0	0	0	0	0
		MIX	25	0	0	0	0	0
Oropharyngeal pain	1	HDmu	26	0	0	0	0	0
		LDmu	26	7.7	0	0	0	7.7
		MIX	26	0	0	0	0	0
		1Dim	26	0	0	0	0	0
		2Dim	26	0	0	0	0	0
	2	HDmu	26	3.8	0	0	0	3.8
		LDmu	26	0	0	0	0	0
		MIX	25	8.0	0	0	0	8.0
Swelling of the pharynx	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	3.8	0	0	0	3.8
		1Dim	26	0	0	0	0	0
		2Dim	26	0	0	0	0	0
	2	HDmu	26	3.8	0	0	0	3.8
		LDmu	26	0	0	0	0	0
		MIX	25	4.0	0	0	0	4.0
Difficulty in pronunciation	1	HDmu	26	0	0	0	0	0
		LDmu	26	0	0	0	0	0
		MIX	26	0	0	0	0	0
		1Dim	26	0	0	0	0	0
		2Dim	26	0	0	0	0	0
	2	HDmu	26	3.8	0	0	0	3.8
		LDmu	26	0	0	0	0	0
		MIX	25	4.0	0	0	0	4.0

*NMPA toxicity grade: see Table S2.

Table S2. Toxicity grading scales for solicited adverse events*

	Grade 1	Grade 2	Grade 3	Grade 4
Fever (Axillary temperature)	37.3 °C ~<38.0 °C	38.0 °C ~<38.5 °C	38.5 °C ~<39.5 °C	≥39.5 °C for more than 3 days
Fatigue	No influence on daily activities	Influence normal daily activities	Seriously influence daily activities and cannot work	Emergency or hospitalization
Headache	No influence on daily activities, no treatment required	Transient, slight influence on daily activities, may require treatment or intervention	Significant disruption of daily activity requiring treatment or intervention	Intractable, requiring emergency room visit or hospitalization
Myalgia (non-vaccination site)	No influence on daily activities	Slight influence on daily activities	Severe muscle pain, severe interference with daily activities	Emergency or hospitalization
Arthralgia	Mild pain, no interference with function	Moderate pain; pain requiring analgesics and/or pain preventing function but not influencing on daily activities	Severe pain; requiring analgesics and/or pain influencing on daily activities	Disabling pain
Nausea	Transient (< 24 h) or intermittent and essentially normal food intake	Sustained nausea leading to reduced food intake (24-48 h)	Persistent nausea resulting in little to no food intake (> 48 h) or need for intravenous hydration	Life-threatening (e.g. hypotensive shock)
Cough	Transient, no treatment required	Persistent cough, effective treatment	Paroxysmal cough that cannot be controlled by treatment	Emergency or hospitalization
Dyspnoea	Dyspnoea on exercise	Dyspnoea with normal activity	Dyspnoea at rest	Dyspnoea requiring oxygen therapy, hospitalization or assisted breathing
Oropharyngeal pain and swelling	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe in severity, severe interference with daily activities, treatment required	NA
Oral mucositis	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA

*According to *Guidelines for grading standards of adverse events in clinical studies of prophylactic vaccines* (No. 102, 2019) issued by the China National Medical Products of Administration, https://www.nmpa.gov.cn/yaopin/ypggtg/ypqtgg/20191231111901460.html.

Table S3. The occurrence of grade 3 fever in participants with different pre-existing Ad5 NAb titers after vaccination

Vaccination	Cohort	Participants with grade 3 fever	Participants not with grade 3 fever	Fisher's exact test P value
Intramuscular vaccination	Pre-existing Ad5 NAb titer≤200（N=36）	6	30	0.0076
	Pre-existing Ad5 NAb titer＞200（N=42）	0	42	
aerosal vaccination	Pre-existing Ad5 NAb titer≤200（N=23）	3	16	0.70
	Pre-existing Ad5 NAb titer＞200（N=29）	4	29	

Table S4. Abnormal laboratory parameters within 7 days after each vaccinations*

Laboratory parameters	HDmu group		LDmu group		MIX group		1Dim	2Dim
	Post prime (n=26)	Post booster (n=26)	Post prime (n=26)	Post booster (n=26)	Post prime (n=26)	Post booster (n=25)	Post prime (n=26)	Post prime (n=26)
Leukocyte Count	0	1	1	2	0	0	0	0
Neutrophil count	0	0	1	2	0	0	0	0
Lymphocytes ratio	0	1	0	0	0	0	0	0
Alanine aminotransferase	0	0	1	0	2	1	0	0
Aspartate aminotransferase	0	1	1	1	1	0	0	0
Fasting blood glucose	0	0	1	1	0	0	0	0
Total bilirubin	1	0	0	0	0	0	0	0

*The laboratory parameters were tested before each dose and 8 days after each dose. All participants were in the normal range of the parameters before the first dose, and the numbers shown in the table are number of participants with abnormal values after the each dose.

Table S5. The geometric mean of RBD-binding IgG, RBD-binding IgA, SARS-CoV-2 neutralising antibody, and Ad5 neutralising antibody, 95% confidence intervals and seroconversions following Ad5-nCoV vaccination

Assay	Study Day		HDmu group		LDmu group		MIX group		1Dim group		2Dim group
		N	GMC or GMT [95% CI], Seroconversion	N	GMC or GMT [95% CI], Seroconversion	N	GMC or GMT [95% CI], Seroconversion	N	GMC or GMT [95% CI], Seroconversion	N	GMC or GMT [95% CI], Seroconversion
RBD-binding IgG	0	26	17 [14, 21], -	26	19 [15, 24], -	26	16 [13, 19], -	26	25 [21, 30], -	26	26 [23, 28], -
	14	26	30 [25, 36], 7.7%	26	44 [37, 51], 11.5%	26	141 [85, 233], 57.7%	26	181 [123, 268], 65.4%	26	210 [128, 343], 61.5%
	28	26	124 [83, 186], 69.2%	26	63 [40, 99], 38.5%	26	960 [603, 1531], 96.2%	26	915 [588, 1423], 92.3%	26	1190 [776, 1824], 96.2%
	42	26	191 [89, 408], 46.2%	26	190 [84, 431], 61.5%	24	1223 [682, 2195], 100.0%				
	56	26	261 [121, 563], 76.9%	26	289 [138, 606], 69.2%	25	2013 [1180, 3435], 100.0%				
RBD-binding IgA	0	26	39 [30, 50], -	26	41 [28, 59], -	26	38 [28, 52], -	26	33 [22, 50], -	26	34 [23, 52], -
	14	26	52 [39, 69], 7.7%	26	114 [84, 155], 34.6%	26	186 [103, 335], 42.3%	26	305 [180, 519], 73.1%	26	341 [181, 643], 69.2%
	28	26	148 [104, 211], 30.8%	26	95 [55,165], 19.2%	26	475 [281, 803], 76.9%	26	425 [231, 784], 73.1%	26	521 [272, 997], 84.6%
	42	26	257 [137, 484], 53.8%	26	265 [127, 554], 50.0%	24	506 [259, 988], 75.0%				
	56	26	312 [153, 634], 50.0%	26	297 [132, 665], 46.2%	25	777 [378, 1601], 76.0%				
SARS-CoV-2 neutralising antibody	0	26	4 [-, -], -	26	4 [-, -], -	26	4 [-, -], -	26	4 [-, -], -	26	4 [-, -], -
	14	26	13 [9, 19], 42.3%	26	11 [8, 16], 30.8%	26	38 [21, 71], 73.1%	26	65 [39, 117], 88.5%	26	83 [51, 134], 92.3%
	28	26	40 [22, 73], 65.4%	26	27 [15, 51], 53.8%	26	73 [46, 117], 92.3%	26	95 [61, 147], 92.3%	26	180 [113, 288], 100.0%
	42	26	118 [50, 280], 84.6%	26	146 [66, 325], 84.6%	24	135 [77, 235], 95.8%				
	56	26	107 [47, 245], 84.6%	26	105 [47, 232], 80.8%	25	396 [207, 758], 100.0%				
Ad5 neutralising antibody	0	26	148 [54, 406]	26	154 [58, 410]	26	131 [48, 359]	26	123[41, 369]	26	231 [78, 689]
	14	26	335 [116, 964]	26	221 [72, 679]	26	1640 [837, 3212]	26	1311 [602, 2854]	26	2400 [1211, 4754]
	28	26	377 [128, 1109]	26	205 [79, 534]	26	1371 [742, 2532]	26	1023 [445, 2347]	26	1547 [702, 3409]
	42	26	779 [269, 2255]	26	450 [151, 1336]	24	2974 [1553, 5698]				
	56	26	936 [311, 2814]	26	400 [144, 1109]	25	2831 [1678, 4777]				

GMC for RBD-binding IgG and IgA, GMT for SARS-CoV-2 and Ad5 neutralising antibody, Seroconversion only for RBD-binding IgG, RBD-binding IgA and SARS-CoV-2 neutralising antibody.

CI= Confidence interval. N=Sample number. GMC=Geometric mean concentrations. Seroconversion (%): The proportion of participants whose antibody titers or concentrations increased by at least four times compared to baseline.

Table S6. Comparison of SARS-CoV-2 neutralising antibody in participants with different gender

Cohort	Visit point	Gender	N	GMT	Lower 95%CI	Upper 95%CI	P value
HDmu group	Day 28	Male	13	65	27	159	0.090
		Female	13	24	10	56	
	Day 56	Male	13	198	57	695	0.13
		Female	13	58	18	183	
LDmu group	Day 28	Male	13	49	22	112	0.063
		Female	13	15	6	38	
	Day 56	Male	13	237	94	599	0.031
		Female	13	46	13	161	
MIX group	Day 28	Male	13	67	30	151	0.71
		Female	13	80	44	145	
	Day 56	Male	12	305	113	828	0.44
		Female	13	504	192	1322	
1Dim group	Day 28	Male	13	119	73	195	0.29
		Female	13	76	35	164	
2Dim group	Day 28	Male	13	194	97	389	0.76
		Female	13	168	81	350	

CI= Confidence interval. N=Sample number. GMT=Geometric mean titre.

Table S7. Comparison of SARS-CoV-2 neutralising antibody in participants with different age

Cohort	Visit point	Age	N	GMT	Lower 95%CI	Upper 95%CI	P value
HDmu group	Day 28	18-55	20	35	17	69	0.41
		⩾56	6	62	12	323	
	Day 56	18-55	20	97	36	261	0.67
		⩾56	6	147	18	1189	
LDmu group	Day 28	18-55	20	27	13	54	0.88
		⩾56	6	30	5	177	
	Day 56	18-55	20	137	54	344	0.21
		⩾56	6	43	6	293	
MIX group	Day 28	18-55	20	94	59	152	0.040
		⩾56	6	32	8	123	
	Day 56	18-55	19	705	386	1289	0.0003
		⩾56	6	64	23	177	
1Dim group	Day 28	18-55	20	93	54	160	0.83
		⩾56	6	103	45	239	
2Dim group	Day 28	18-55	20	197	112	348	0.49
		⩾56	6	134	47	382	

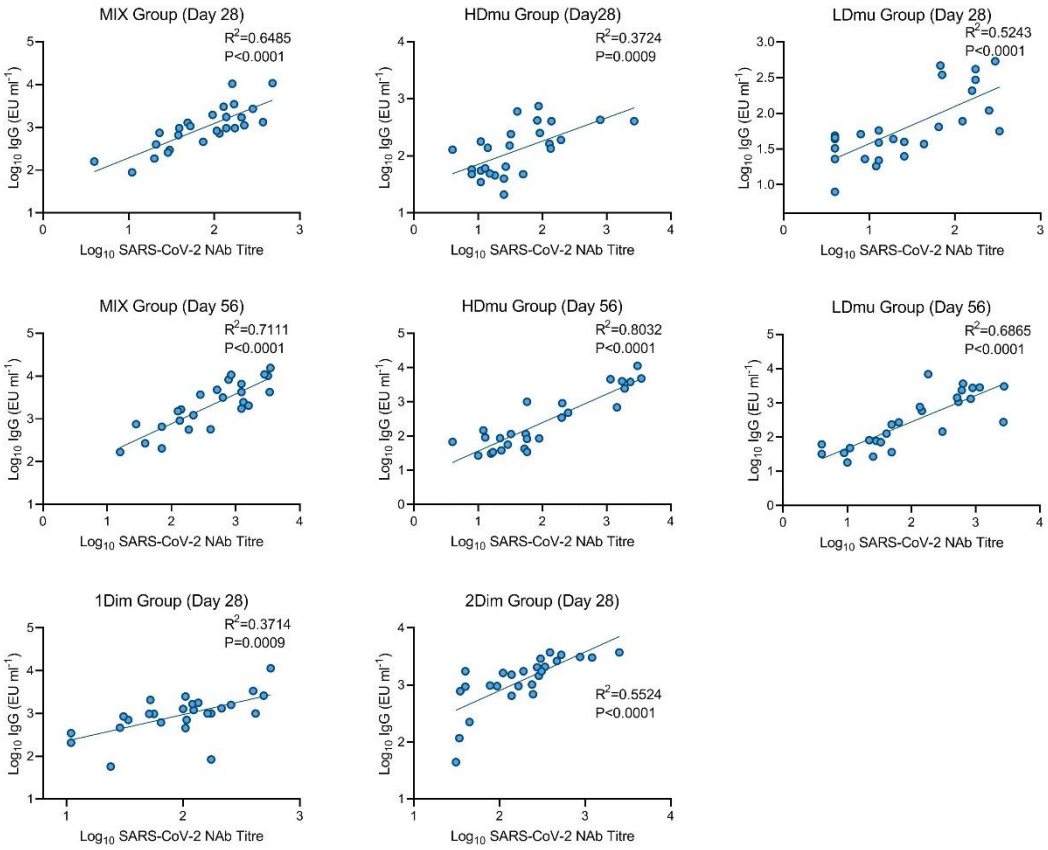
CI= Confidence interval. N=Sample number. GMT=Geometric mean titre.

Table S8. Comparison of SARS-CoV-2 neutralising antibody in participants with different pre-existing Ad5 neutralising antibody

Cohort	Visit point	Ad5 neutralising antibody	N	GMT	Lower 95%CI	Upper 95%CI	P value
HDmu group	Day 28	≤200	12	100	37	267	0.0018
		>200	14	18	11	30	
	Day 56	≤200	12	482	141	1652	0.0010
		>200	14	29	16	53	
LDmu group	Day 28	≤200	11	77	31	193	0.0052
		>200	15	13	7	24	
	Day 56	≤200	11	485	184	1282	0.0001
		>200	15	34	15	78	
MIX group	Day 28	≤200	12	132	70	246	0.014
		>200	14	45	24	83	
	Day 56	≤200	12	564	227	1397	0.29
		>200	13	286	104	791	
1Dim group	Day 28	≤200	13	178	109	290	0.0014
		>200	13	51	28	90	
2Dim group	Day 28	≤200	11	356	194	651	0.0078
		>200	15	110	60	201	

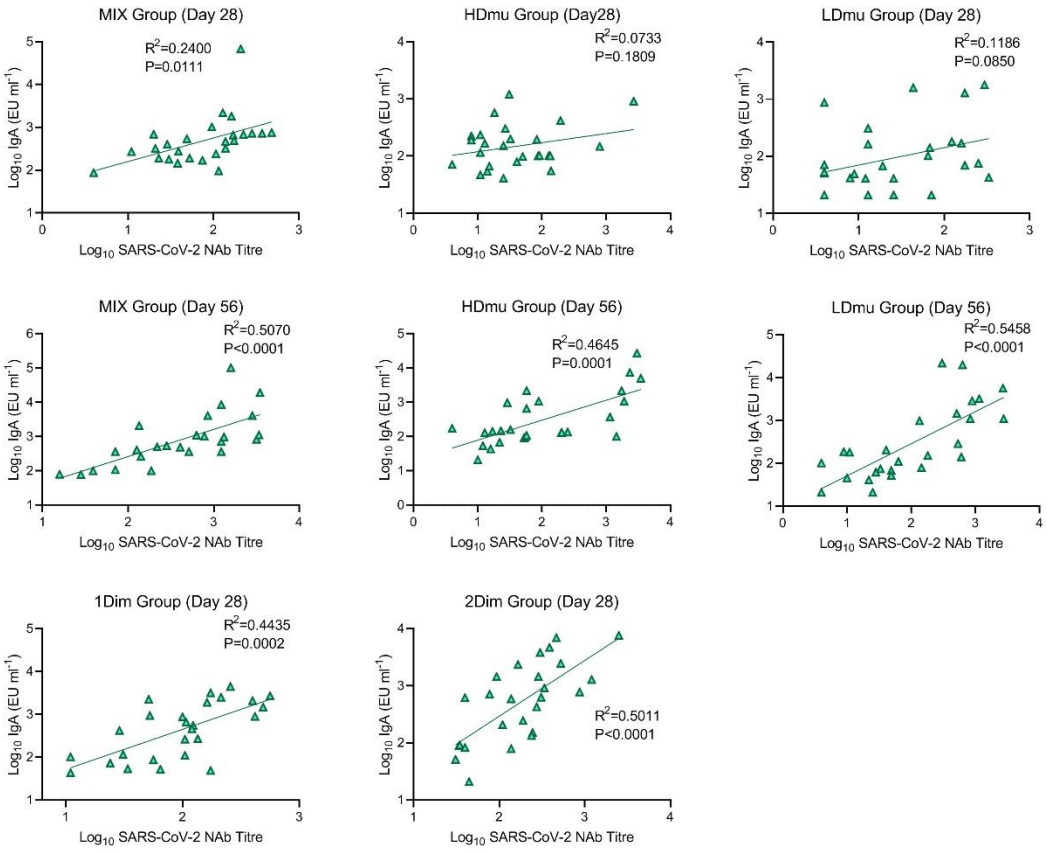
CI= Confidence interval. N=Sample number. GMT=Geometric mean titre.

Figure S1. Correlates of RBD-binding IgG concentrations and SARS-CoV-2 neutralising antibody titres



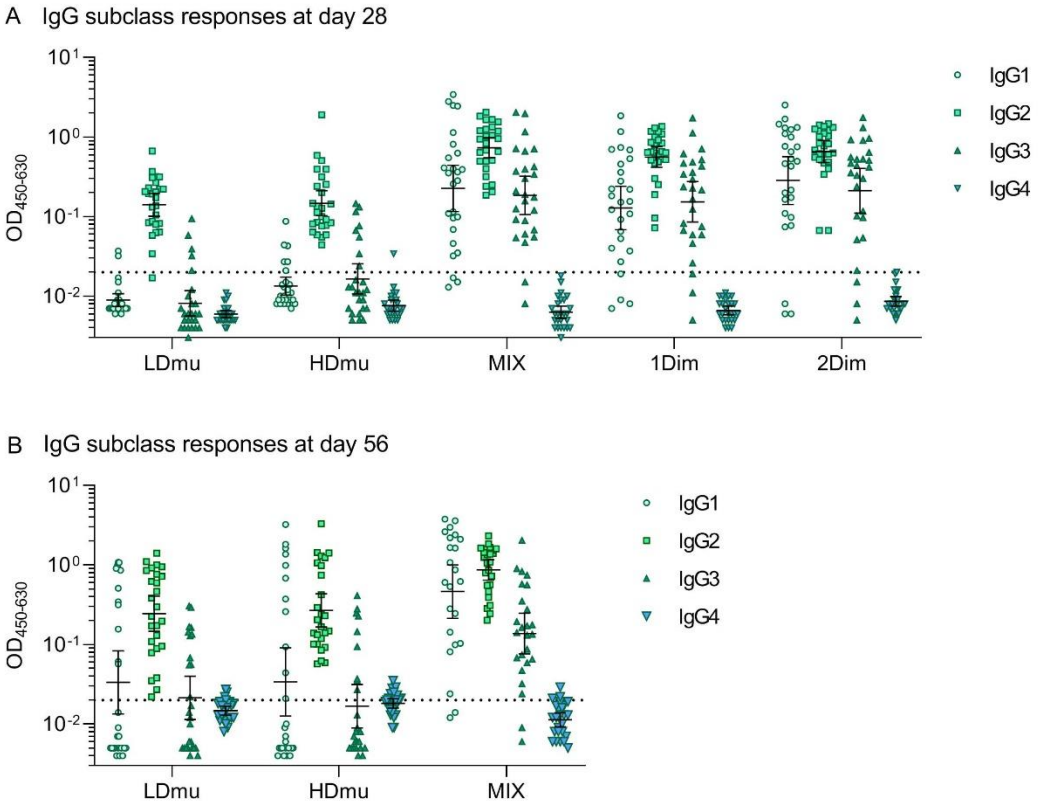
Pearson's correlation coefficient was calculated among RBD-binding IgG concentrations and SARS-CoV-2 neutralising antibody titres at day 28 and day 56 after initial vaccination.

Figure S2. Correlates of RBD-binding IgA concentrations and SARS-CoV-2 neutralising antibody titres



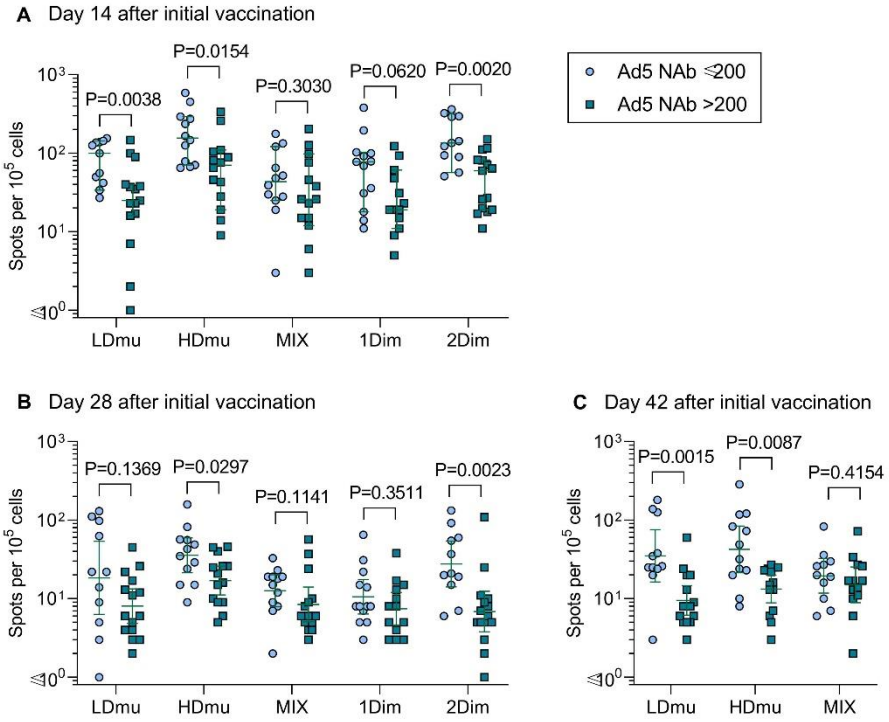
Pearson's correlation coefficient was calculated among RBD-binding IgA concentrations and SARS-CoV-2 neutralising antibody titres at day 28 and day 56 after initial vaccination.

Figure S3. IgG subclass responses induced by Ad5-nCoV vaccination



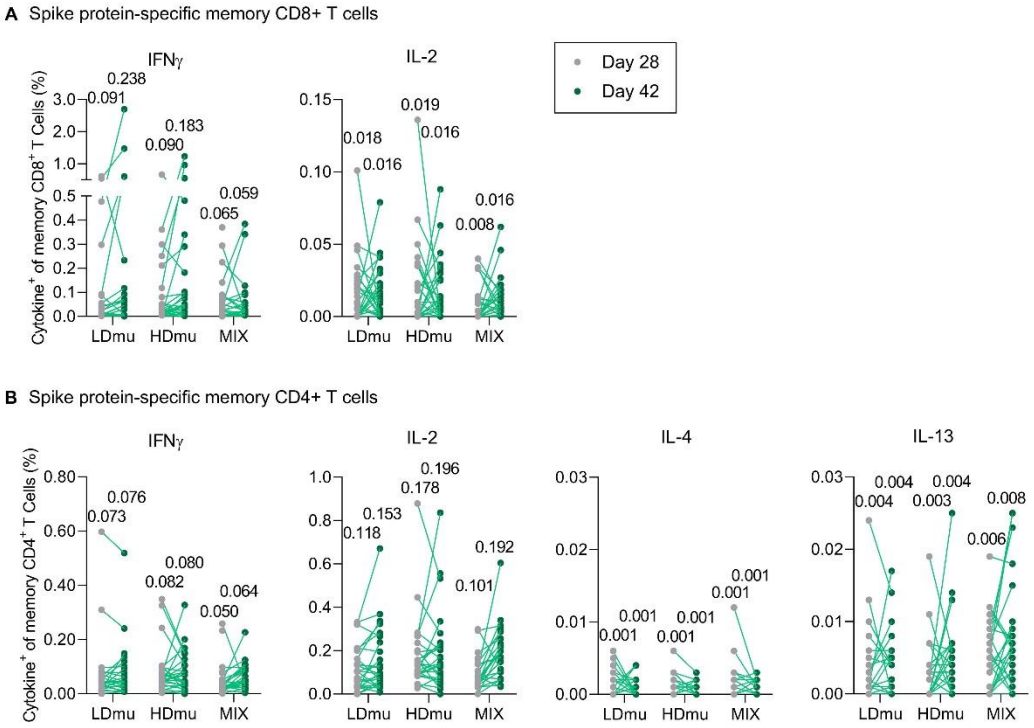
Serum samples at day 28 (A) and day 56 (B) were assayed for IgG subclasses by ELISA and expressed as OD values. Each data point represents a serum sample, and each vertical bar represents the geometric mean with 95% confidence interval. Dotted line indicate the lower limit of quantification (LLOQ). IgG1 data of two samples in the MIX group on day 56 are not included due to exceeding the detection limit.

Figure S4. IFNγ response stratified by pre-existing Ad5 neutralising antibody titers



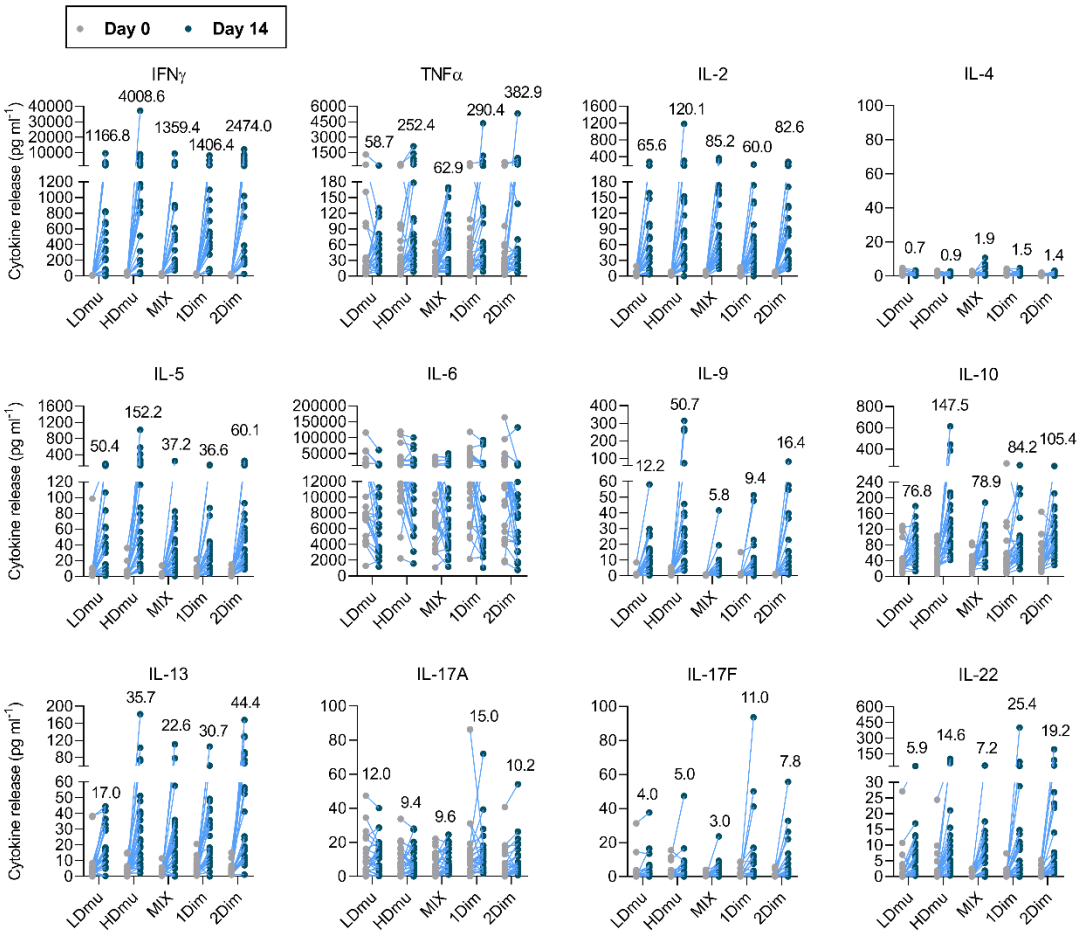
SRAS-CoV-2 spike -specific IFNγ were detected by ELISpot and stratified by pre-existing Ad5 neutralising antibody titers (titer of >1:200 vs ≤1:200). Each data point represents the average number of spots from triplicate wells for one participant, after subtraction of the unstimulated control. Each vertical bar represents median with 95% confidence interval. Significant difference was determined by two-tailed nonparametric Mann–Whitney's rank test.

Figure S5. Memory CD4+ and CD8+ T cell response of Ad5-nCoV before and after booster vaccination



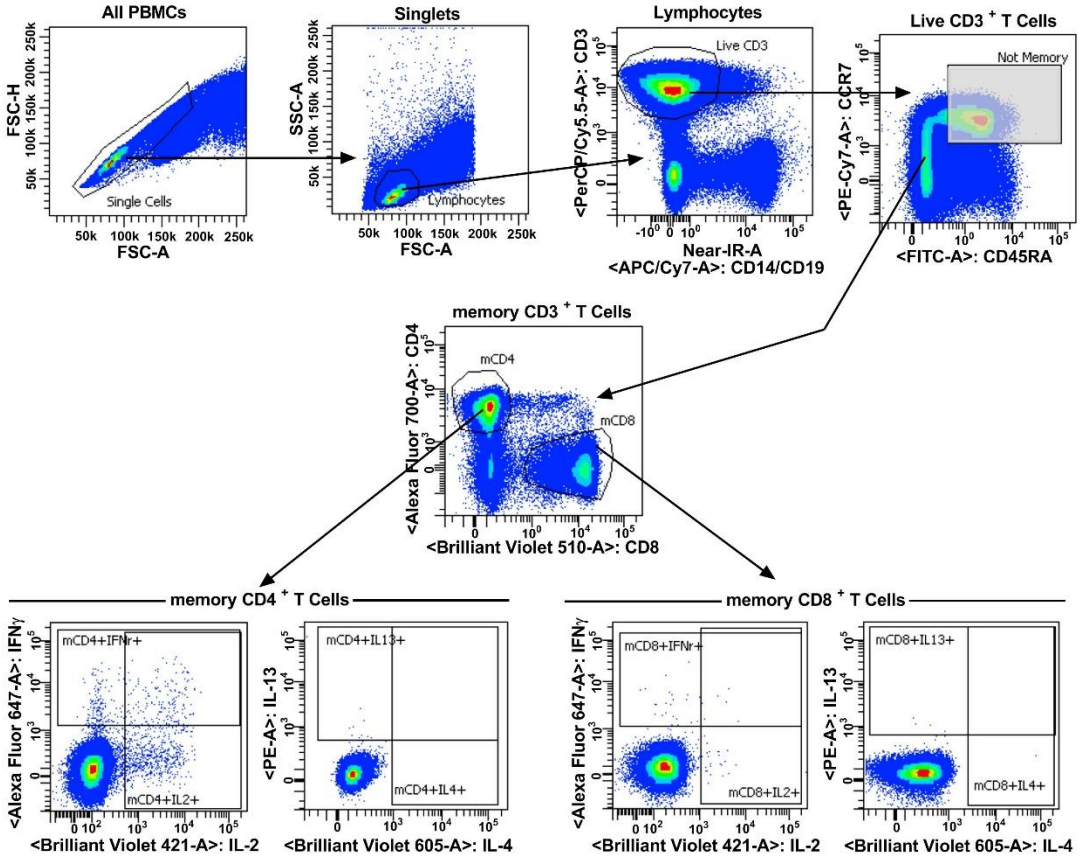
Freshly isolated PBMCs from HDmu, LDmu and MIX groups on day 28 (before boost) and day 42 (14 days after boosting) were stimulated for 6 hours with a SARS-CoV-2 spike protein peptide pool and analyzed by flow cytometry. The gating strategy is depicted in Figure S7. A, Spike -specific IFNγ and IL-2 responses in memory CD8+ T cells. B, pike protein-specific IFNγ, IL-2, IL-4 and IL-13 responses in memory CD4+ T cells. Each data point represents the average percentage of indicated cytokine in T cell subsets from duplicate tests, after subtraction of the unstimulated control. The number above the data points are mean values for the group.

Figure S6. Th cytokines in the supernatants of peptide pool stimulated PBMCs before and after prime vaccination



Freshly isolated PBMCs on day 0 and day 14 were stimulated for 24 hours with a SARS-CoV-2 spike protein peptide pool, and the supernatants were collected for detection the concentrations of IFNγ, TNF-α, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F and IL-22 by a bead-based Th cytokine multianalyte flow assay kit. Each data point represents the concentration of indicated cytokine after subtraction of the DMSO control. The number above the data points are mean values for the group.

Figure S7. Gating strategy of intracellular cytokine staining flow cytometry



Stimulated PBMCs were stained and analyzed by flow cytometry. The sample was progressively gated to identify single cells, lymphocytes, live CD3+ T cells, memory CD3+ T cells and memory CD4+ or memory CD8+ T cells as shown in the top and middle row. Gates for IFNγ, IL-2, IL-4 and IL-13 were created within both memory CD4+ and CD8+ T cells (bottom).

Immunogenicity study of two doses of Recombinant novel coronavirus vaccine (adenovirus vector)

Project Number：AMMS85-2004

Study conducted by: Zhongnan Hosptial of WuHan University

Sponsor: Institute of Biotechnology, Academy of Military Medical Sciences, China

Version Number ： Version 1.1

Version Date ： 27 September, 2020

Synopsis

Short title	Immunogenicity study of two doses of recombinant novel coronavirus vaccine (adenovirus vector)
Title	Immunogenicity and safety study of two doses of a recombinant novel coronavirus vaccine (adenovirus vector)via intramuscular injection, mucosal vaccination, or combination of intramuscular injection/mucosal vaccination in healthy adults aged 18 years and older.
Study purpose	To assess immunogenicity and safety of two doses of a recombinant novel coronavirus vaccine (adenovirus vector)via intramuscular injection, mucosal vaccination, or combination of intramuscular injection/mucosal vaccination in adults aged 18 years and older
Indication	Prevention of COVID-19 causing by SARS-CoV-2
Population	Healthy adults aged 18 years and older.
Sample size	144 subjects
Rational	SARS-CoV-2 virus is enveloped, nonsegmented, positive-sense single-stranded RNA virus genome, which belongs to the orthocoronavirus subfamily of coronaviridae in Nidovirales. There are six types of coronaviruses known to infect humans, including 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV). SARS-CoV-2 virus belongs to a novel kind of β coronavirus, with common symptoms in upper respiratory tract and few in systems. COVID-19, caused by SARS-CoV-2, has been an epidemic disease spreading all over the world. As of 31, Aug 2020, over 800,000 deaths have been reported. The recombinant novel coronavirus vaccine (adenovirus vector)(hereafter referred as Ad5-nCoV) has been developed by the Institute of Biotechnology, Academy of Military Medical Science jointly with the CanSino Biologics Inc., in order to prevent COVID-19 caused by the infection of SARS-CoV-2. The Ad5-nCoV is a recombinant replication-defective adenovirus type 5 expressing the SARS-CoV-2 Spike glycoprotein by amplification and purification. Preclinical studies indicate both humoral immunity and cellular immunity play important roles in protective immunity. In March 16 of 2020, phase I study of the Ad5-nCoV vaccine was launched in Wuhan. Total 108 subjects were randomized into low dose, medium dose and high dose groups (36 subjects per each group) to receive 5×10^{10}vp、1×10^{11}vp and 1.5×10^{11}vp vaccines, respectively. Safety data found the vaccine was generally safe in low and medium dose group and tolerated in high dose group. The majority of adverse

	events were mild or moderate in severity and similar with Ad5-EBOV Ebola Vaccine. The vaccine showed good immunogenicity profile by inducing humoral and cellular immunity rapidly. T- cell response and IgG titers peaked on Day14 and Day28, respectively. In April 12 of 2020, phase II study of the Ad5-nCoV vaccine was launched in Wuhan. Total 508 subjects were randomized into medium dose, low dose group and placebo group at a ratio of 2:1:1. Good safety and high-level immunogenicity profile were found in subjects receiving low and medium dose of vaccine. The Ad5-nCoV vaccine has received special military drug approval of the Health Bureau of the Logistics Support Department under the Central Military Commission in June 25, 2020. SARS-CoV-2 challenge study indicated the vaccine had better protective effect on the upper respiratory tract of animal models by mucosal Immunization, compared with intramuscular injection (IM) (Wu, et al. Nature Communications, (2020) 11:4081). The study aims to further understand the immune response induced by Ad5-nCoV vaccine through mucosal vaccination in human body. Based on preliminary data of phase I and II studies, adults aged 18 years and older in this study will receive 2 doses of Ad5-nCoV vaccine by IM, mucosal vaccination or combination of IM/mucosal vaccination to further evaluate the immunogenicity and safety characters of the vaccine.
Candidate vaccine	**Vaccine:** Generic name: Recombinant novel coronavirus vaccine (adenovirus vector) **Developed by:** Institute of Biotechnology, Academy of Military Medical Sciences and CanSino Biologics Inc. **Specification**: 0.5mL/ vial, 5×10^{10}vp **Dosage：** Mucosal vaccination: high dose 2×10^{10}vp（0.2ml）; low dose 1×10^{10}vp（0.1ml）; Intramuscular injection: 5×10^{10}vp（0.5ml） **Route of Administration：** Mucosal vaccination: using respiratory mucosal immune device. Intramuscular injection: lateral deltoid of the upper arm **Storage and transportation**: Stored and transported at +2°C to +8°C in a dark place, protected from freezing strictly.

Study design

Study design:

Subjects in the study will be randomized and stratified by route of administration, vaccination schedule (A group excluded). Total 144 subjects will be enrolled into 6 parallel groups randomly: A group , 2 IM doses with 56 days apart (24); C group, 2 doses of IM and mucosal immune(high dose) with 28 days apart (24); D group, 2 doses of mucosal immune(high dose) with 28 days apart (24); E group, 2 doses of mucosal immune(low dose) with 28 days apart (24); F group, 1 IM dose as a contrast (24); G group, 2 IM doses in right and left arms simultaneously. Subjects will randomized into 6 groups listed above as a ratio of 1:1:1:1:1:1, stratified by sex (Male/ Female) and age (18~55 years, and ≥ 56 years).

Randomization and blood sampling schedule are listed in the following table:

Group	Sample size	Route of administration		Schedule	Procedure	Note
		1st dose	2nd dose			
A	24	IM	IM	Day0,56	Before and 28 days after 1st dose，before and 14 days, 28days and 6 months days after 2nd dose	People who had been inoculated with intramuscular injection of 1 dose
C	24	IM	Mucosal vaccination	Day0,28	Before and 14 days after 1st dose，before and 14days, 28days and 6 months days after 2nd dose	High dose for mucosal vaccination
D	24	Mucosal vaccination	Mucosal vaccination	Day0,28		High dose
E	24	Mucosal vaccination	Mucosal vaccination	Day0,28		Low dose
F	24	IM	--	Day0	Before and 14 days, 28 days, 6 months after vaccination	--
G	24	IM	IM	Day0	Before and 14 days, 28 days, 6 months after vaccination	Left,right arm for each dose
Total	144					

	Infection during the study: During the observation period of the study, subjects with persistent fever, along with cough or other respiratory symptoms, should go to the designated hospital immediately, and inform the researcher. Nasopharyngeal swabs/ sputum should be collected and detected. CT and other imaging examinations should be performed to determine whether the symptoms were caused by SARS-CoV-2 infection. If COVID-19 cases are confirmed during the study, case investigation should be carried out, with backup blood sample tested for infection. Critical or death cases need to be investigated further on an individual basis. **Study duration:** Subjects will be followed for about 8 months from enrollment to last visit. Some subjects may terminate the study in advance.
Study endpoints	**Primary endpoints:** **1. Safety endpoint** Incidence of adverse events (AE) for 0-7 days post each vaccination **2. Immunogenicity endpoints** Geometric Mean Concentration (GMC) and seroconversion rates of SARS-CoV-2 Spike RBD-specific antibody levels (ELISA) 28 days post full vaccination Geometric Mean Titre (GMT) and seroconversion rates of SARS-CoV-2 neutralizing antibody levels 28 days post full vaccination **Secondary endpoints** **1. Safety endpoint** Incidence of adverse events (AE) for 30/60 minutes (30 min for IM, 60 min for mucosal) post vaccination Incidence of adverse events (AE) for 8-28 days post each vaccination Incidence of serious adverse events (SAE) occurring from 1st dose until 6 months post full vaccination **2.Immunogenicity endpoints** ①GMC and seroconversion rate of SARS-CoV-2 Spike RBD-specific antibody levels (ELISA) detected before 1st dose of all groups, before 2nd dose (F, G excluded), 14 days post 1st or 2nd dose; ②GMT and seroconversion rate of SARS-CoV-2 neutralizing antibody detected before 1st dose of all groups, before 2nd dose (F, G excluded), 14 days post 1st or 2nd dose; ③IFN-γ level (ELISpot) detected before 1st dose of all groups,14 days post vaccincation (A excluded), before (F, G excluded) and 14 days post 2nd dose. ④ SARS-CoV-2 neutralizing antibody against AdHu5 detected before 1st dose of all groups, before 2nd dose (F, G excluded), 14 days and 28 days post 2nd dose; ⑤GMI of SARS-CoV-2 Spike RBD-specific antibody (ELISA) detected before 1st dose of all

	groups, before 2nd dose (F, G excluded), 14 days and 28 days post 2nd dose; ⑥For A group: GMC, seroconversion rate and GMI of SARS-CoV-2 Spike RBD-specific antibody (ELISA) detected 28 days post 1st dose; GMT and seroconversion rate of SARS-CoV-2 neutralizing antibody (pseudovirus) post 1st dose; SARS-CoV-2 neutralizing antibody against AdHu5. Note: Seroconversion rate is defined as: proportion of subjects with ≥4-fold rises in GMT between pre-vaccination and specific days after final vaccination. **Exploratory endpoints**: ① GMC of SARS-CoV-2 Spike RBD-specific antibody (ELISA), SARS-CoV-2 Spike-specific antibody (ELISA) and GMT of neutralizing antibody against AdHu5 detected 6 months post full vaccination ② IgA antibody levels detected before 1st dose of all groups (A, G excluded), 14 days post 1st dose, 14 days and 28 days post 2nd dose; ③ T cell specific cytokines and other mucosal immune-related characteristics detected by flow cytometry.
Study procedure	**Group A:** 8 visits, including V0 (1st vaccination day), V1(28 +3 days post 1st dose), screening visit (-3 days to the 2nd vaccination day), V2 (56+5 days post 1st dose / 2nd vaccination day), V3 (8+1 days post 2nd dose), V4 (14+1 days post 2nd dose), V5 (28+3 days post 2nd dose), V6 (6 months± 15 days post full vaccination). ① V0 (1st vaccination day): informed consent form signed, blood collection at baseline, vaccination, observation after vaccination; ② V1 (28 +3 days post 1st dose): observation of adverse events, blood collection; ③ Screening visit (-3 days to the 2nd vaccination day): Confirm written informed consent has been obtained, screening, check inclusion and exclusion criteria, exclusion of ineligible subjects; ④ V2 (56+5 days post 1st dose / 2nd vaccination day): assignment of study number, blood collection, vaccination, observation after vaccination, issue Diary card; ⑤ V3 (8+1 days post 2nd dose): observation of adverse events, collection of Diary card, issue contact card; ⑥ V4 (14+1 days post 2nd dose): blood collection, observation of serious adverse events; ⑦ V5 (28+3 days post 2nd dose): blood collection, observation of adverse events, collection of contact card; ⑧ V6 (6 months± 15 days post full vaccination): collection of blood, observation of serious adverse events. **Group C/D/E:** 9 visits, including screening visit (-3 day to -1 day before V0), V0 (1st vaccination day), V1(8 +1 days post 1st dose), V2 (14+2days post 1st dose), V3 (28+3 days post 1st dose / 2nd vaccination day), V4 (8+1 days post 2nd dose), V5 (14+2 days post 2nd dose), V6(28+3 days post 2nd dose), V7(6 months± 15 days post full vaccination).

	① Screening visit (-3 day to -1 day before V0): Confirm written informed consent has been obtained, screening, check inclusion and exclusion criteria; ② V0 (1st vaccination day): randomization, blood collection at baseline, collection of nasopharyngeal swabs, vaccination, observation after vaccination, issue Diary card (Day 0-7); ③ V1 (8 +1 days post 1st dose): laboratory test for safety, observation of adverse events, collection of Diary card, issue Contact card; ④ V2 (14+2 days post 1st dose): blood collection, collection of nasopharyngeal swabs, observation of serious adverse events; ⑤ V3 (28+3 days post 2nd dose/ 2nd vaccination day): collection of Contact card, blood collection, collection of nasopharyngeal swabs, laboratory test for safety, vaccination, observation after vaccination, issue Diary card; ⑥ V4 (8+1 days post 2nd dose): laboratory test for safety, observation of adverse events, collection of Diary card, issue Contact card; ⑦ V5 (14+2days post 2nd dose): collection of blood and nasopharyngeal swabs, observation of serious adverse events; ⑧ V6(28+3 days post 2nd dose): collection of blood and nasopharyngeal swabs, observation of adverse events, collection of Contact card (Day 8-28); ⑨ V7 (6 months± 15 days post full vaccination): collection of blood, observation of serious adverse events; **Group F/G:** 6 visits, including screening visit (-3 day to -1 day before V0), V0 (1st vaccination day), V1(8 +1 days post 1st dose), V2 (14+2days post 1st dose), V3 (28+3 days post 1st dose), V4(6 months± 15 days post full vaccination). ① Screening visit (-3 day to -1 day before V0): Confirm written informed consent has been obtained, screening, check inclusion and exclusion criteria; ② V0 (1st vaccination day): randomization, blood collection at baseline, collection of nasopharyngeal swabs (G excluded), vaccination, observation after vaccination, issue Diary card; ③ V1 (8 +1 days post 1st dose): laboratory test for safety, observation of adverse events, collection of Diary card, issue Contact card; ④ V2 (14+2 days post 1st dose): blood collection, collection of nasopharyngeal swabs (G excluded), observation of serious adverse events; ⑤ V3 (28+3 days post 2nd dose/ 2nd vaccination day): blood collection, collection of nasopharyngeal swabs (G excluded), observation of adverse events, collection of Contact card; ⑥ V4 (6 months± 15 days post full vaccination): collection of blood, observation of serious adverse events.
Criteria for	

suspension or premature Termination	Adverse events after vaccination will be collected and reported to DSMB every week (All SAE should be reported immediately). All safety data will be reviewed by DSMB independently based on weekly report. Sponsor, principle investigator, ERCs/IRBs or regulatory authority have right to suspend or terminate the study if violations of the protocol, GCP requirements or ethical requirements happens. Reasons should be explained to other parties and Subject. If one of the following situations occurs, the sponsor will convene an expert panel meeting involving investigators and DSMB, to determine whether to early terminate the clinical study: ① Any subject experience a Grade 4 adverse event that is considered related to vaccination by investigators; ② Any subject experience an SUSAR; ③ Occurrence of grade ≥3 adverse events that lasts for at least 48 hours in more than 20% of subjects. ④ The study has a large potential safety risk assessed by DSMB; **The study will be terminated if one of the following situations occurs:** ① Sponsor decides to discontinue development of the study vaccine and explains the reason; ② ERCs/IRBs decides to discontinue development of the study vaccine and explains the reason; ③ Regulatory authority decides to discontinue development of the study vaccine and explains the reason.
Statistical analysis	**Interim analysis:** Statistical analysis and study report will be conducted when last Subject finish visit of 28 days post full vaccination. **Final analysis:** Final analysis will be conducted when last Subject finish last visit : 6 months post full vaccination.
Inclusion criteria	Adults aged 18 years and older; Be capable of signing the informed consent forms; Be able and willing to comply with study protocol and complete the follow-up Have negative result for HIV screening; Axillary temperature ≤37.0℃ Have negative result for SARS-CoV-2 specific antibodies (IgG and IgM) detection(A group excluded) Eligible for the study after obtaining details and results of medical history, physical examination and laboratory test.
Exclusion criteria	**Exclusion criteria:** Individual with abnormal laboratory test, or with clinically significant abnormalities judged by investigators (including, WBC count, lymphocyte count, neutrophils, platelets, hemoglobin, alanine aminotransferase ALT, aspartate aminotransferase AST, total bilirubin, fasting glucose, creatinine). (A group excluded);

	Individual with oral ulcer, swollen throat and other oral diseases; Individual with upper respiratory tract infection; Medical history or family history of convulsions, epilepsy, encephalopathy and psychosis; History of allergy to any components of the vaccine or serious allergic reaction; Individual with acute febrile diseases and infectious diseases; History of laboratory-confirmed SARS-CoV-2 infection; Previous treatments for curing COVID-19 or vaccination of COVID-19 vaccines; Severe cardiovascular diseases, e.g, arrhythmia, conduction block, myocardial infarction, severe hypertension and uncontrollable hypertension with medication (SBP ≥ 140mmHg, DBP≥ 90mmHg); Serious chronic disease or in a progressive condition that cannot be controlled well, such as asthma, diabetes, thyroid disease, etc.; Congenital or acquired angioedema/ neuroedema; Have urticarial in the past 1 year; Congenital or functional absence of spleen; Have thrombocytopenia or other blood coagulation disorders (Intramuscular injection is not allowed); Individual with fainting during acupuncture treatment (in IM groups); Receipt of immunosuppressant therapy, anti-allergic therapy, cytotoxic therapy, inhaled corticosteroid aerosol in the past 6 months (Surface corticosteroid therapy for acute non-complicated dermatitis excluded); Receipt of blood product within 4 months prior to administration of study vaccine; Receipt of other study drugs within 1 month prior to administration of study vaccine; Receipt of live attenuated vaccines within 1 month prior to administration of study vaccine; Receipt of subunit vaccines or inactivated vaccines within 14 days prior to administration of study vaccine; Current treatment of anti-TB; Women with positive pregnancy test, lactating women, or have plan to be pregnant within 6 months; Ineligible for the study based on the assessment of the Investigators, including protocol deviation, or unavailable for signing the informed consent form. **Exclusion criteria of 2nd Vaccination:** Severe allergic reactions occurring after 1st vaccination; Severe adverse events related to vaccination occurring after 1st vaccination; New found failure of inclusion/ exclusion criteria, decided by investigator whether to be in the study; Ineligible for the study based on the assessment of the Investigators.
Exclusion criteria for A	Subjects in A group who have received 1st vaccination, completed medical history screening and lab tests, will be excluded if not meeting inclusion / exclusion criteria judged by

group	investigator.
Role of Sponsor	Sponsor will develop the study protocol with investigator, but not participate in other procedures of the study, including data collection, statistical analysis, interpretation of results, as well as writing and review of study report.
Sponsor	Institute of Biotechnology, Academy of Military Medical Sciences, China. Address ： 20 Dongdajie, Fengtai District, Beijing,100071 Contact: Dr. Lihua Hou E-mail：houlihua@sina.com
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Laboratory2	Institute of Biotechnology, Academy of Military Medical Sciences, China. Address ： 20 Dongdajie, Fengtai District, Beijing,100071 Contact: Dr. Jun Zhang E-mail：justforhere@126.com
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Abbreviation

Abbreviation	Full Name
AE	Adverse Event
AR	Adverse Reaction
Ad5	Replication Defective Human Adenovirus Serotype 5
COVID-19	Corona Virus Disease 2019
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMI	Geometric Mean Fold Increase
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titre
IEC	Independent Ethics Committee
ITT	Intent-to-treat
NIFDC	National Institute for Food and Drug Control
NMPA	National Medical Products Administration
PPS	Per Protocol Set
SAE	Serious Adverse Event
SOP	Standard Operation Procedure
SS	Safety Set
vp	Virus Particle

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1. Purpose and introduction

The novel recombinant coronavirus vaccine (Adenovirus vector), namely Ad5-nCoV developed by the Institute of Biotechnology, Academy of Military Medical Sciences jointly with the CanSino Biologics Inc., is developed to prevent the novel coronavirus disease (COVID-19) caused by the infection of SARS-CoV-2.

Pre-clinical animal studies demonstrated that the Ad5-nCOV had good immunogenicity profile in BALB/c mice, guinea pigs, ferrets and rhesus monkeys. In challenge study, the candidate vaccine provided protection against SARS-CoV-2 in animal models. Additionally, safety data found the candidate vaccine was generally safe. Safety data of 28 days post vaccination in phase I study showed that, candidate vaccine was generally safe in low and medium dose group and well tolerated in high dose group. The majority of adverse events were mild or moderate in severity and similar to the Ad5-EBOV Ebola Vaccine (Adenovirus vector). The candidate vaccine showed good immunogenicity profile by inducing humoral and cellular immunity rapidly. T- cell response and IgG titers peaked on Day14 and Day28, respectively.

Phase II study of the candidate vaccine was launched in Wuhan in 12, Apr 2020. 508 subjects were randomly divided into medium, low and placebo groups at the ratio of 2:1:1. Favorable safety profile were observed in both low and medium dose groups, with low dose group better than medium dose group. Humoral immunogenicity data showed that the seroconversion rate (SRC) by anti-S RBD-binding antibodies was high in both low and medium dose groups, with SRC > 95% at 28 days after vaccination. In both low and medium dose groups, the SRC of neutralizing antibody measured by pseudovirus neutralization test were > 80% at 28 days after vaccination; Cellular immunogenicity data showed that, the proportion of specific IFN -γ positive detected by ELISpot or neutralizing antibody measured by wild-type assay in both low and medium dose groups were greater than 90%.

Based on the safety and immunogenicity results of phase I clinical study, as well as the safety results of phase II clinical study, 2 doses of candidate vaccine via mucosal vaccination or combination of IM/ mucosal vaccination with 28 days interval will be conducted with expanded population, adults aged 18 years and older. This I /II study of mucosal vaccination approach will further assess the immunogenicity and safety profile of candidate vaccine.

The clinical study protocol is designed according to the requirements of the "Vaccine management law", "Drug registration management measures", "Drug clinical study quality management standard" (GCP), "Vaccine clinical study technical guidelines" and "vaccine clinical study quality management guidelines" (study).

2. Study site

Zhongnan Hospital of Wuhan University

3. Clinical study related units

3.1 Sponsor

Institute of Biotechnology, Academy of Military Medical Sciences, China.

3.2 Investigator

Zhongnan Hospital of Wuhan University

3.3 CRO – Clinical monitoring

Shanghai STEM Pharmaceutical Development Limited

3.4 CRO- Statistical and Data Management

Shanghai STEM Pharmaceutical Development Limited

3.5 Research Laboratory

Zhongnan Hospital of Wuhan University
Institute of Biotechnology, Academy of Military Medical Sciences, China.

3.6 Data Safety Monitoring Committee

Sponsor is responsible for organizing this committee.

4. Background and Rationale

4.1 Pathogeny

The 2019 novel coronavirus disease (COVID-19) is a contagious disease caused by the infection of the novel coronavirus (SARS-CoV-2). SARS-CoV-2 virus belongs to the beta genus of coronavirus, with envelop and the particles are round or oval shape, usually are polymorphous, with a diameter of 60-140 nm. The gene characteristics of SARS CoV-2 is significantly different with SARS CoV and MERS-CoV. It is reported by Chinese scientists that SARS-CoV-2 shares 88% of sequence identity to two types of bats (bat-SL-CoVZC45 and bat-SL-CoVZXC21) in Zhoushan, China. The SARS-CoV-2 is the seventh coronavirus that is capable of infecting human beings, and it has never been discovered before.

The coronavirus belongs to coronaviridae and coronavirus in systematic taxonomy. Coronavirus is a kind of positive-strand RNA virus with envelope, and a kind of virus widely existing in nature. 10%-30% of upper respiratory tract infections are caused by four kinds of coronaviruses: HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 in the world. It is the second leading cause of common cold, after rhinovirus. Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) caused by coronavirus are known as serious infectious diseases. The coronavirus genome encodes spike protein (S), envelope protein (E), membrane protein (M) and nucleoprotein (N) in turn. S protein is the most important surface protein, which has an essential role in virus entry into the targeted cell. S protein contains two subunits: S1 and S2, S1 mainly contains receptor binding region which is responsible for the recognition of cell receptor; S2 contains the basic elements required for membrane fusion process. S protein was emerged as the most important candidate antigen in prior vaccine development of SARS and MERS.

4.2 Background of disease and epidemiology

Fever, dry cough and fatigue are major manifestations of the disease. A small number of patients have nasal congestion, runny nose, sore throat, myalgia, diarrhea and other symptoms. Severe patients usually have dyspnea and / or hypoxemia one week after the onset of the disease, the more severe patients could quickly progress to acute respiratory distress syndrome, septic shock, incorrigible

metabolic acidosis, hemorrhage, coagulation dysfunction, and multiple organ failure. It is worth noting that SARS-CoV-2 causes mild or moderate fever, or asymptomatic fever in severe and critical patients. For some children and newborns, SARS-CoV-2 may causes atypical symptoms, including diarrhea, vomiting and other gastrointestinal symptoms, or only mental weakness and shortness of breath. Mild patients are only associated with low fever and slight fatigue, with no symptoms suggestive of pneumonia. Based on the available data, most patients had good prognosis, and a few patients were in critical condition. The prognosis is poor for the elderly and the people with chronic diseases. For pregnant women with COVID-19, is similar to others in same age group. The symptoms of children with COVID-19 are relatively mild.

The major source of infection is SARS-CoV-2 infected patients. Asymptomatic patient also cause infection. Respiratory droplets and close contact are the main routes of transmission. Exposure to high concentrations of aerosols for a long time in a relatively closed environment may cause aerosol transmission. SARS-CoV-2 can be isolated from feces and urine, therefore attention should be paid to the spread of aerosols or contacts caused by feces and urine. Both men and women are generally susceptible to SARS-CoV-2.

4.3 Background

4.3.1 Recombinant nCOV-19 vaccine (Adenovirus vector) (Ad5-nCoV)

The novel recombinant coronavirus vaccine (Adenovirus vector)(briefly as candidate vaccine) developed by Institute of Biotechnology, Academy of Military Medical Sciences jointly with the CanSino Biologics Inc., is based on the mature platform of recombinant replication-defective human adenovirus type 5 vector, which is able to effectively express the targeted antigen(S protein) of SARS-CoV-2 in transfected/infected cells. The candidate vaccine is supposed to protect subjects through eliciting both humoral and cellular immunogenicity responses, as well as mucosal vaccination response against S protein of SARS-CoV-2.

4.3.2 SARS-CoV-2 virus vaccines in development

The novel coronavirus vaccines currently being developed are mainly in the following categories: inactivated vaccine: has complete virus structure with no pathogenicity, maintaining total or partial immunogenicity. After vaccination, the virus antigen can stimulate the body to elicit immune response and provide the protection. Inactivated vaccines are generally prepared through the following steps: firstly, the virus strains should be cultured and screened on suitable cells to obtain the virus with high immunogenicity, titer and stability, which can be used to establish the seed bank for large-scale production of vaccines in the future. After that, candidate vaccine will be fostered, inactivated and

purified with relatively simple process. It is one of the traditional and classic methods of vaccine preparation. The main obstacles lie in two aspects: firstly, pathogenic and immunological mechanisms of SARS-CoV-2 virus is not fully understood, and inactivated whole virus may carry some harmful components; secondly, at present, cultivation of live SARS-CoV-2 virus requires biosafety level 3 conditions, which limit the production capability of inactivated vaccines.

Recombinant subunit vaccine: has subunit antigen(s) of virus stimulating the body to produce protective immunity against COVID-19. This kind of vaccine has favorable safety but relatively low immunogenicity with small size. So, new techniques and adjuvants are often used in this platform to increase vaccine's immunogenicity. Design and construct of subunit vaccine, as well as evaluation of its efficacy play key roles in its research& development cycle.

Adenovirus vector vaccine: replication-deficient adenovirus vector vaccine carrying 2019 nCoV antigen gene which can efficiently express the target antigen of 2019 nCoV in transfected / infected cells, so that the body produces corresponding humoral and cellular immunity against diseases caused by 2019 nCoV. The novel recombinant vaccine based on replication-deficient human adenovirus 5 developed by the sponsor has now progressed to phase III clinical study. Meanwhile, a novel chimpanzee adenovirus vaccine developed by Oxford University / AstraZeneca (ChAdOx1 nCoV-19) has entered the phase III clinical study stage.

Attenuated influenza virus vector vaccine: the vaccine is inoculated by nasal drip. The inoculation efficiency will be enhanced if this kind of vaccine is developed successfully. As of reporting date, there are no reports of similar vaccines yet in other countries in the world.

mRNA vaccine: mRNA encoding multiple antigens from key targets of 2019-nCoV will be synthesized in vitro and delivered to the body cell for translating into antigen protein, which would stimulate the body's immune system and induce specific immune response. It is easy to produce and modify mRNA vaccine, with high efficiency and relatively low cost. But this kind of vaccine is unstable and usually elicits strong immunogenicity. Currently, most mRNA vaccines are in the clinical stage, and not approved by regulatory authority yet. At present, mRNA-1273 from Moderna Therapeutics and BNT162b1 from Pfizer / BioNtech have entered phase III clinical studies. China's self-developed mRNA vaccine has also entered phase I clinical study recently.

4.4 Advantages of candidate vaccine

The effective component of the vaccine is a replication-deficient recombinant adenovirus (Ad5-nCoV) expressing gene encoding spike protein of 2019-nCoV. In this project, the candidate vaccine has been prepared in large-scale and been studied in quality control according to GMP requirements. It also has been evaluated in pharmacodynamics and toxicology. Animal experiments data showed that the vaccine can stimulate humoral and cellular immunity in vivo. The main features of the vaccine are as following: 1. Strong pertinence or immunogenicity profile: this vaccine is designed based on antigen sequence of 2019-nCoV, and is targeted well for the

prevention ofCOVID-19 causing the expanding global epidemic. 2. Mature technology platform: this vaccine shares same adenovirus vector technology platform with approved Ad5-EBOV Ebola Vaccine (Adenovirus vector). The platform has standardized production process and sophisticated quality control system. 3. It is easy to produce on a large scale. The technology is mature, which can meet the needs of large-scale population usage.

5 Pre-clinical and prior clinical studies

5.1 Pre-clinical immunogenicity assessments

5.1.1 Mice

BALB/c mice were immunized with low, medium and high dose of Ad5-nCoV via intramuscular injection (IM) and mucosal injection (IN), with empty adenovirus vector as negative control. Samples were collected at different time points to measure humoral and cellular immune responses specific to Ad5-nCov vaccine. The levels of anti-S IgG binding protein, neutralizing antibody measured by wild-type assay and pseudo-neutralization assay were detected before vaccination, 14 days, 28 days, 42 days, and 56 days after vaccination. T-cell response in spleen and antibodies in bronchial alveolar lavage fluid were detected 14 days after vaccination in other 3 groups administered medium dose of Ad5-nCoV or Ad5 vector via IM and IN.

5.1.1.1 Anti-S IgG binding antibody detection results

The results showed that Ad5-nCoV showed good immunogenicity by two routes of administration:

anti-S IgG binding antibody peaked at day 28 after inoculation by IM, and then decreased slightly; anti-S IgG binding antibody peaked at day 28 after inoculation by IN and remained stable until day 56; in high dose group, anti-S IgG binding antibody titer of IM group was higher than that of IN group (42 days, $P < 0.0001$, 56 days $P=0.0001$). In medium and low dose groups, there was no significant difference in IgG antibody titer between two routes of administration at day 42 and day 56 after inoculation ($P > 0.05$).

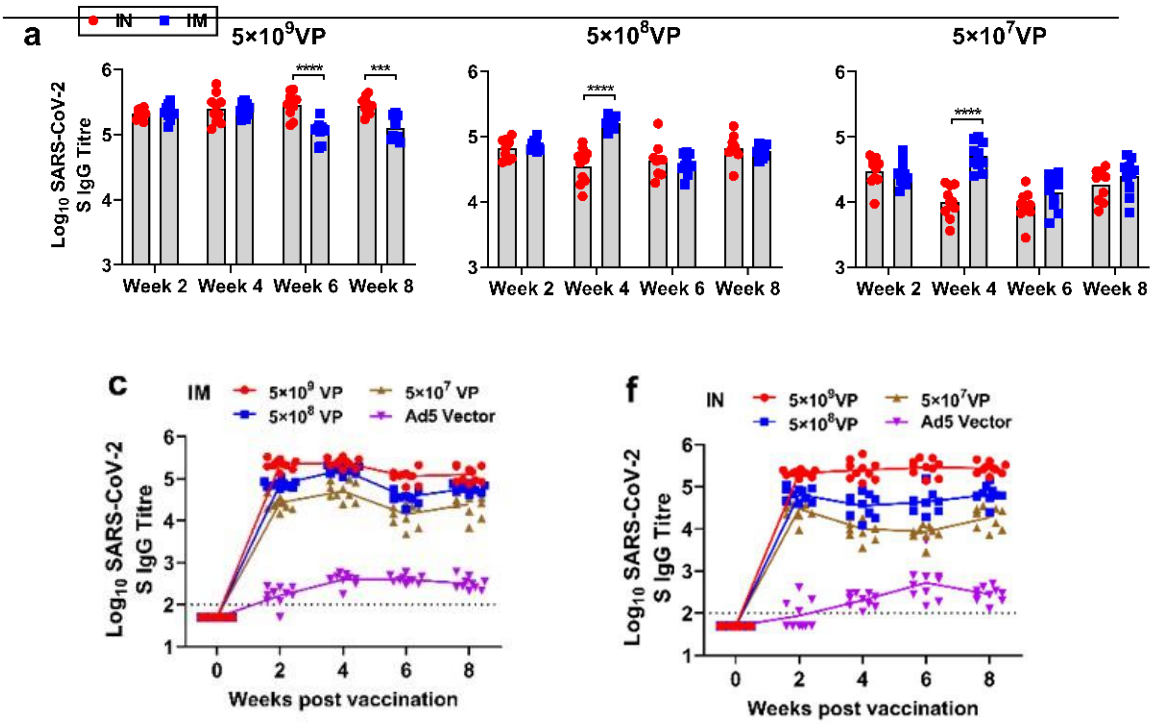


Figure 5-1-1-1 Levels of anti-S IgG binding antibody of mice at Day14, Day28, Day42 and Day56 after single immunization

At 14 days and 10 weeks after immunization, anti-S IgG binding antibody was detectable in bronchial alveolar lavage fluid in both IM and IN groups. anti-S IgA binding antibody was only detected in IN group.

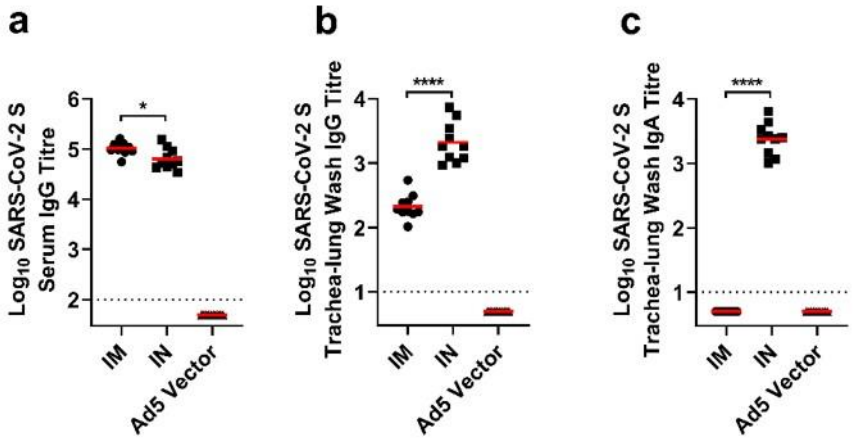


Figure 5-1-1-2 Levels of anti-S IgG and IgA binding antibody in serum and bronchial alveolar lavage fluid of mice after single immunization

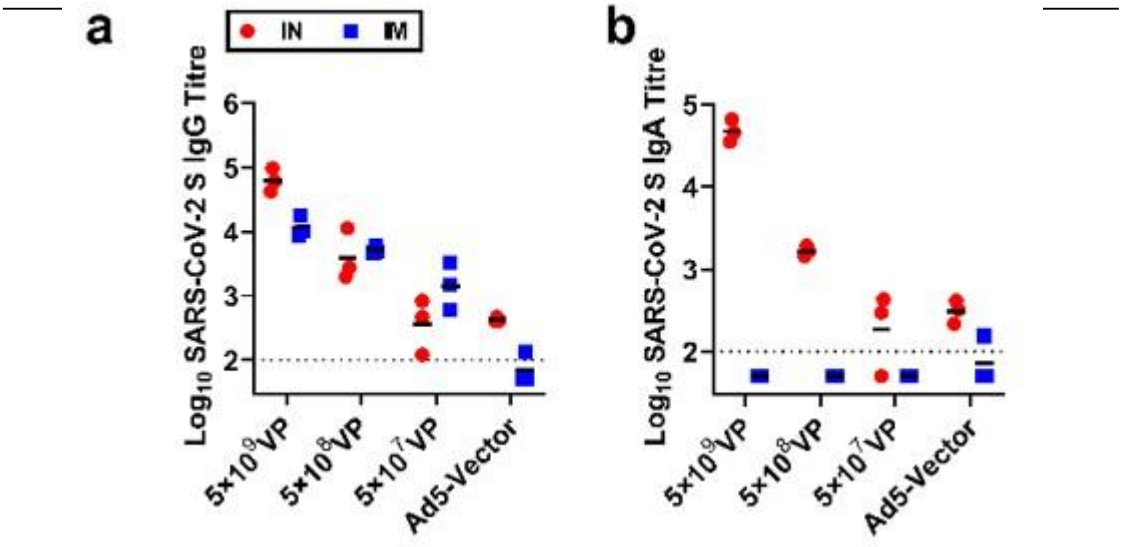
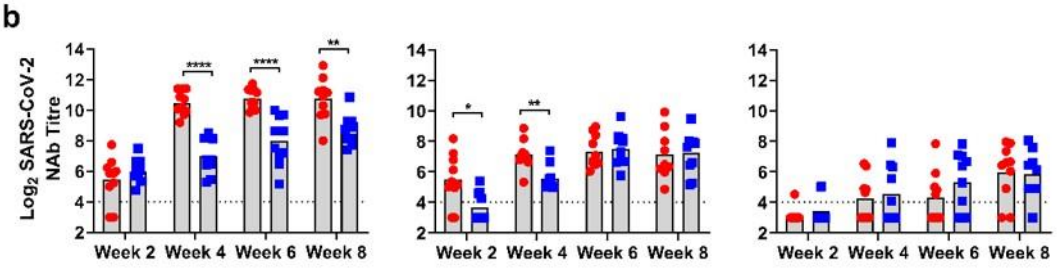


Figure 5-1-1-3 Levels of anti-S IgG and IgA binding antibody in bronchial alveolar lavage fluid of mice 10 weeks after immunization

5.1.1.2 Neutralizing antibody detection results

The levels of serum anti-SARS-CoV-2 neutralizing antibody (NAb) were measured by microcytopathic effect inhibition assay at Day14, Day28, Day42 and Day56 after single intramuscular injection and mucosal immunization.

The results showed that Ad5 nCoV showed good immunogenicity by two routes of administration: neutralizing antibody peaked at Day42 after inoculation by mucosal immunization, while peaked at Day56 after inoculation by intramusculr injeciton; in high dose group, neutralizing antibody titer by mucosal immunization was significantly higher than that by intramuscular injection from Day28 to Day56 after inoculation (Day 28, P<0.0001; Day 42, P<0.0001; Day56, P=0.0021). There was no significant difference in neutralizing antibody titer between two routes of administration in medium dose group at Day 42 and Day 56 after inoculation. No significant differences were found in neutralizing antibody titer between two routes of administration in low dose group at each time point after inoculation.



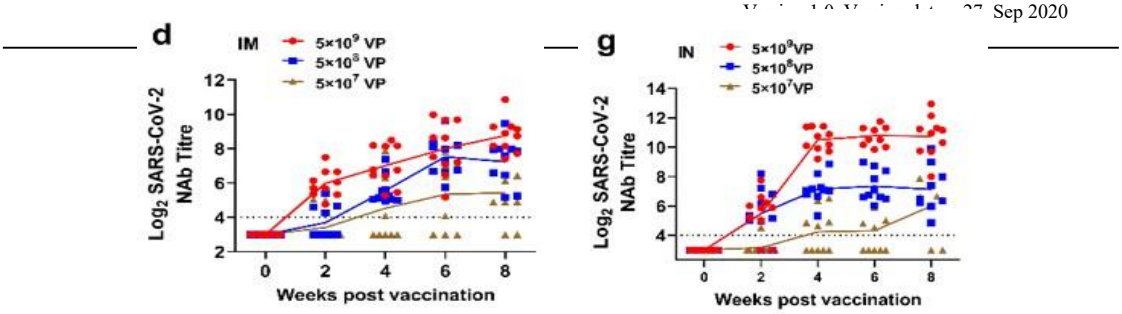


Figure 5-1-1-4 Levels of SARS-CoV-2 neutralizing antibody at Day14, Day28, Day42, and Day56 after single immunization in mice models

Neutralizing antibody measured by pseudo-neutralization assay were detected at 14 days,28 days and 56 days after single inoculation by IM or IN (Refer to the figure below): levels of neutralizing antibody measured by wild-type assay and pseudo-neutralization assay were comparable.

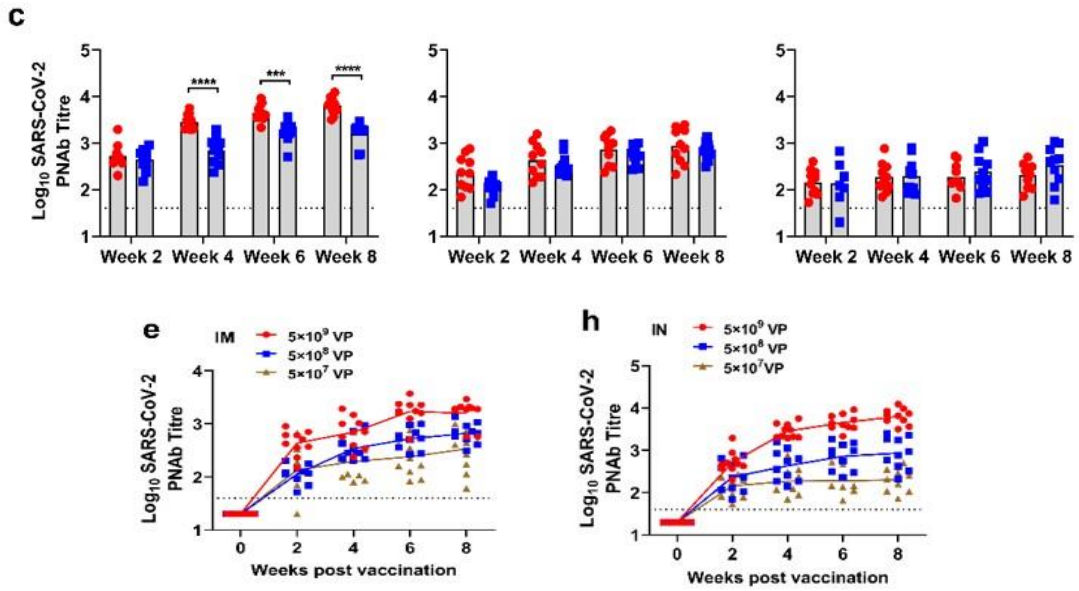


Figure. 5-1-1-5 results of neutralizing antibody against pseudovirus in mice at days of 14, 28, 42 and 56 after single immunization

Neutralizing antibody measured by wild-type assay and pseudo-neutralization assay were detected in bronchial alveolar lavage fluid after inoculation by two routes of administration in high dose group. For low dose group, no neutralizing antibody were detected by either route.

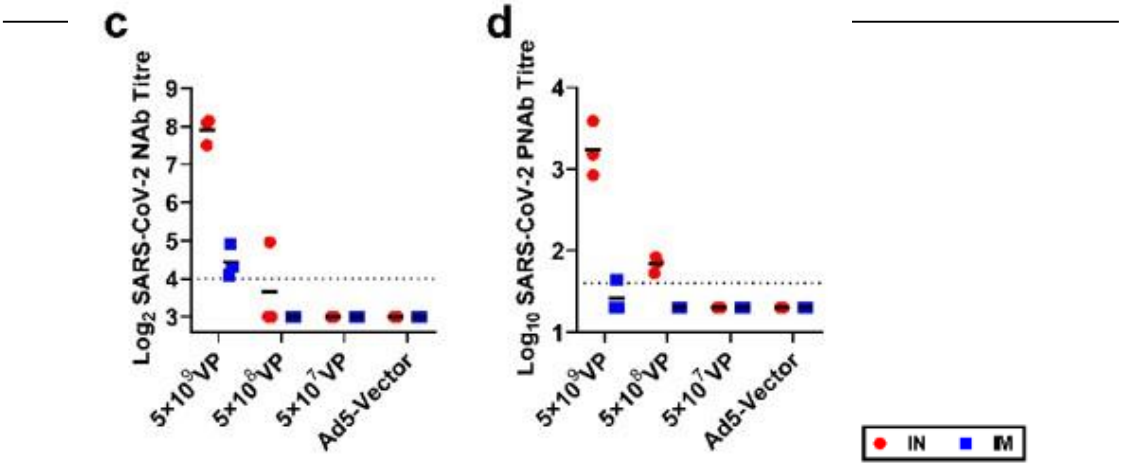


Figure. 5-1-1-6 Levels of neutralizing antibody measured by wild-type assay and pseudo-neutralization assay in bronchial alveolar lavage fluid at 10 weeks after immunization in mice models

Anti-S IgG binding antibody response correlated strongly with neutralizing antibody measured by wild-type assay and pseudo-neutralization assay at Day42 and Day56 after inoculation.

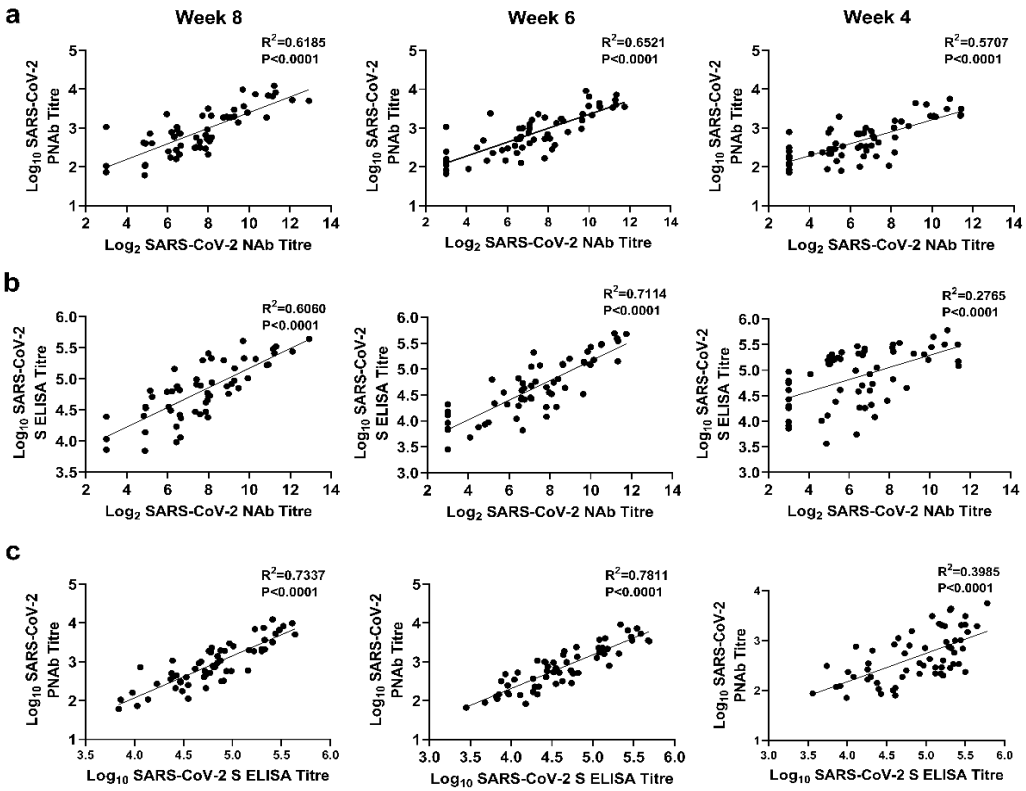


Figure 5-1-1-7 Correlations among IgG binding antibody , neutralizing antibody and neutralizing antibody measured by pseudo-neutralization assay in mice models immunized with Ad5-nCoV

5.1.1.3 Cellular Immune Response

In medium dose group, both intramuscular injection and mucosal immunization could significantly induce the production of IFN - γ, TNF - α and IL-2 by CD8 + and CD4 + T cells 14 days after immunization, which was higher in the intramuscular injection group than in the mucosal immunization group.

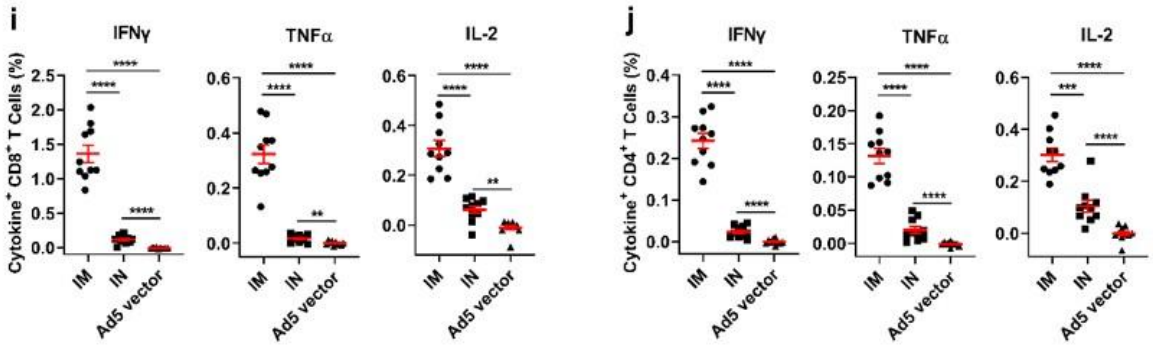


Figure 5-1-1-8 Cellular immune response 14 days after single immunization in mice models

There was a dose-dependent cellular immune response in the intramuscular injection group 10 weeks after immunization. No such response was found in mucosal immunization group.

5.1.2 Ferret model

5.1.2.1 Anti-S IgG binding antibody & neutralizing antibody detection results

Eighteen ferrets were randomly divided into three groups: intramuscular injection group, nasal drip group and negative control, 6 in each group. The anti-S IgG binding antibody and neutralizing antibody were detected in all vaccinated groups at 28 days after immunization, but not in the control group. There was no significant difference between the two routes of administration.

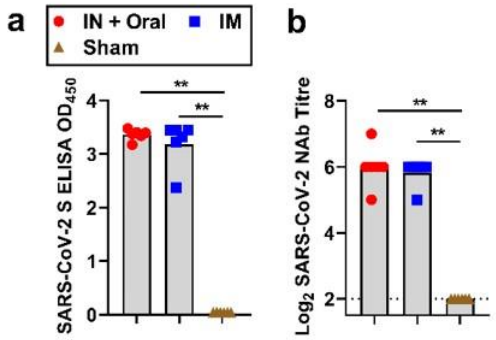


Figure 5-1-2-1 Levels of anti-S IgG binding antibody and neutralizing antibody in ferret models.

5.1.2.2 Cellular Immune Response

IFN-γ - producing T cell response (ELISpot) were detectable in 5 ferrets of intramuscular group and 3 ferrets of mucosal immunization group at 28 days after immunization.

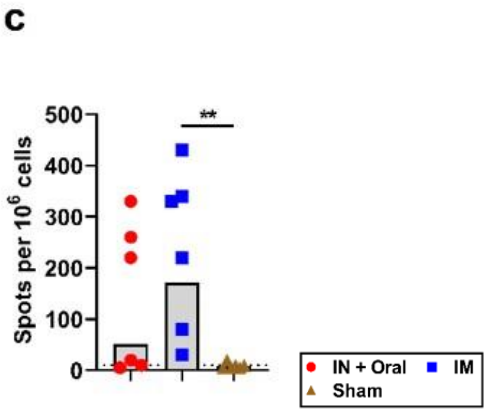


Figure. 5-1-2-2 Cellular immune response in ferrets

5.2 Animal protective experiment

5.2.1 Study on the protective effect in mice model

After 10 weeks of immunization in the mice of immunogenicity study mentioned above, 3 out of 10 mice in each group were euthanized to detect T cell response in spleen and neutralizing antibody in bronchoalveolar lavage fluid. The remaining mice (7 in each group) were challenged by intranasal inoculation of SARS-CoV-2 HRB26M strain at a dose of 103.6 PFU,

50 ml. For each route group, 4 mice were euthanized at 3 days after challenge, theremain 3 mice were euthanized at 5 days after challenge to detect viral load in lung and turbinate.

Virus load was measured by Real-Time qPCR (RT-qPCR)and PFU assay. For mucosal immunization group, no virus was detected in the lung and turbinate of mice euthanized 3 or 5 days after challenge. While in mice euthanized 3 days after challenge in control group, viral load was detectable, with 1.2×10^4 PFU/g in turbinate and 5.6×10^5 PFU/g in lung on average.

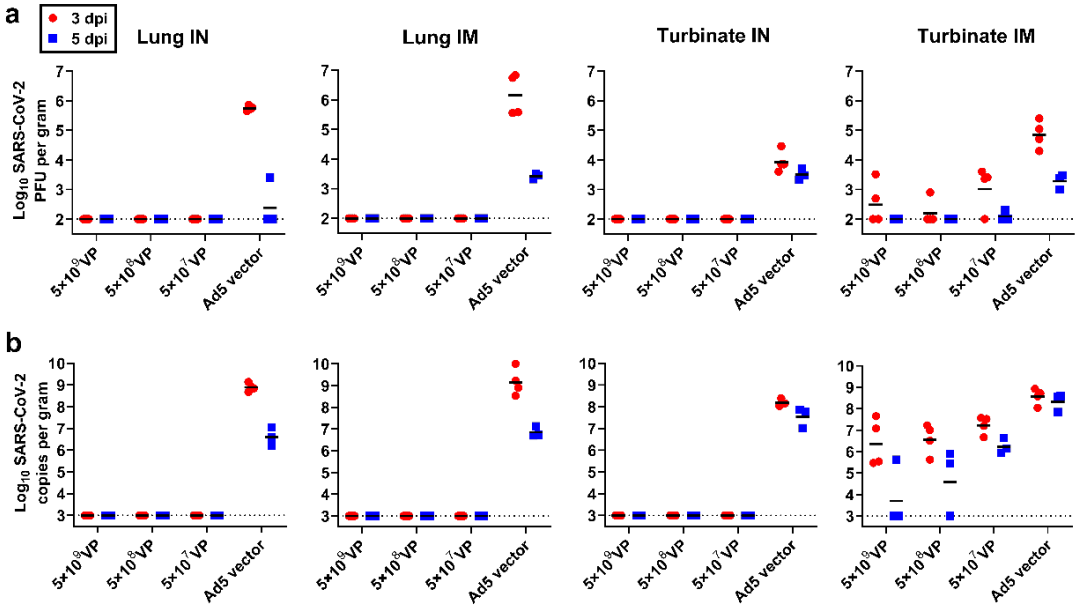


Figure 5-2-1-1 Number of live virus (a) and viral load (b) in lung and turbinate of mice

No virus was detectable in the lung of IM group at 3 and 5 days after challenge. The average viral load was 3.3×10^6 PFU / g in the lung of IM control group at 3 days after challenge. PFU and qPCR were used to detect the virus in the turbinate of some mice in IM group. Compared with the IM control group, the viral load in the turbinate of mice inoculated in three groups was significantly reduced.

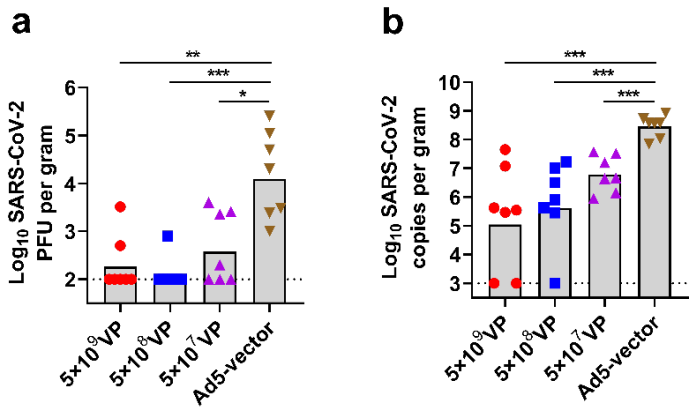


Figure. 5-2-1-2: Viral load in turbinate of mice inoculated by IM after challenge
a. 3 days; b. 5 days

The results showed that a single, low-dose of Ad5 nCoV could completely protect the mice from virus infection in upper respiratory tract and lung. It was more difficult to protect the upper respiratory tract from virus infection than the lower respiratory tract in mice by different inoculation routes.

5.2.2 Study on the protective effect in ferret model

Ferret is a mammalian model. SARS-CoV-2 can replicate in its upper respiratory tract, but not in its lung. Efficacy of Ad5-nCoV vaccine was investigated on the upper respiratory tract of ferrets by mucosal immunization or intramuscular injection to assess its protection against SARS-CoV-2 infection.

At 2~8 days after challenge, no virus was detected in the nasal lavage fluid of mucosal immunization group by qPCR and PFU assay, while all the ferrets in control group were infected. On the 2nd, 4th, 6th and 8th day after challenge, the virus was detected in 3/6(3 out of 6 ferrets), 2/6(2 out of 6 ferrets), 0/6(0 of 6 ferrets) and 0/6(0 of 6 ferrets) of ferrets' nasal lavage fluid by PFU, respectively. Compared with the control group, the viral load of IM group was lower significantly at each time point.

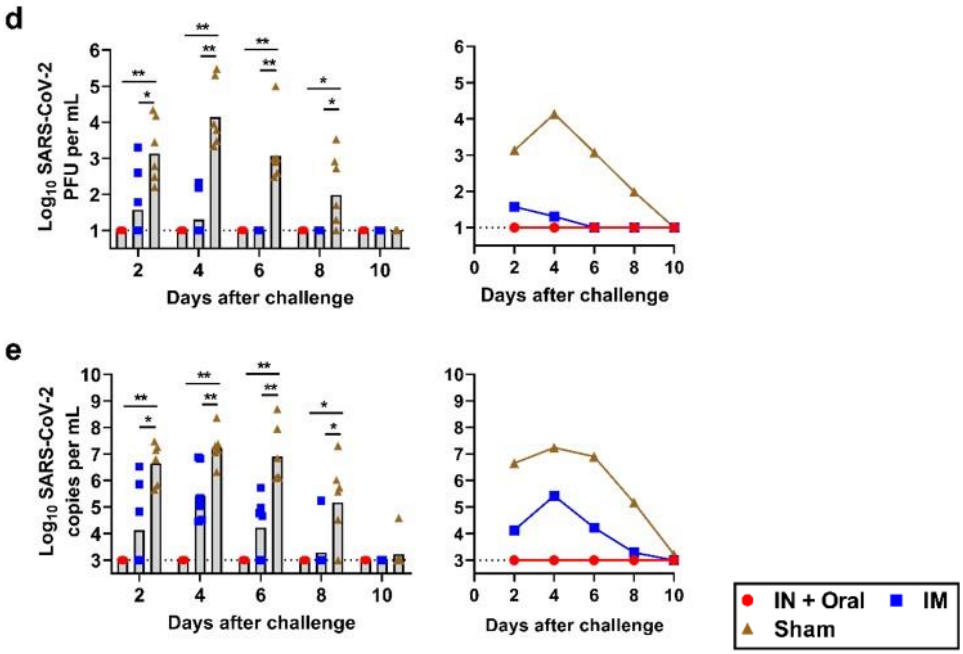


Figure 5-2-2-1 The number of live SARS-CoV-2 virus (d) and viral load (e) in the nasal lavage fluid of ferrets after challenge.

5.3 Pre-clinical toxicity study

5.3.1 Single-dose toxicity study by IM in SD rats

For 1 dose / rat (0.5 x10^{11}vp/dose) group and negative control, no death or dying, adverse clinical signs, abnormal changes in body weights and food consumption were observed. There was no obviously abnormal change in the gross anatomy of the rats in each group, so the histopathological examination was not performed.

In this study, SD rats were administered with single dose of Ad5-nCoV (0.5 x10^{11} vp) by IM. No signs of toxicity were observed. The maximum tolerated dose (MTD) was greater than or equal to 0.5 x10^{11} vp/ dose/rat. IgG antibody against adenovirus vector and anti-S IgG binding antibody were detectable in 2 groups at 2 weeks after administration.

5.3.2 Repeated dose toxicity study by IM in cynomolgus monkeys (2-week administration and 2-week recovery)

No death or dying were observed during the study. No adverse clinical signs were related with the vaccine. No allergic reactions were observed in clinical observation after two doses of vaccine. Compared with the same sex negative control, no obvious changes or toxicological abnormal reaction were observed in low dose (1 dose / animal) and high dose groups (3 doses / animal) in the following indicators: body weight and weight gain, body temperature, ECG waveform and parameters, blood pressure, ophthalmic examination, clinical pathology (blood cell count, coagulation function, blood biochemistry, urine analysis), T lymphocyte subsets (CD3+, CD4 +, CD8 +, CD4 + / CD8 +), serum cytokines (IL-2, IL-4, IL-5, IL-6, TNF - α, IFN - γ), C-reactive protein, serum complement (C3, C4), organ weight, visceral body ratio and visceral brain ratio.

5.3.3 Acute toxicity study by intranasal instillation in SD rats

No death, dying or adverse clinical signs were observed during the study in 1 dose / rat (0.5×10^{11}vp/dose) group and negative control. No obvious changes in body weights and food consumption related with vaccine were observed. There was no obvious abnormal change in the gross anatomy of the rats in each group, so the histopathological examination was not performed. No lgG antibody against adenovirus vector and anti-S lgG binding antibody were detectable in negative control 14 days (Day 15) after single dose of vaccine; In vaccine group, no lgG antibody against adenovirus vector was detectable, while anti-S lgG binding antibody was detected in relatively high level with titer ranging from 1:254666 to 1:409600.

Under the condition of this experiment, SD rats were administered with repeated dose of Ad5-nCoV ($0.5 \text{ x}10^{11}$ vp) by intranasal instillation. No signs of toxicity were observed. The maximum tolerated dose (MTD) was greater than or equal to $0.5\text{x}10^{11}$ vp/ dose (1 dose /rat). Anti-S IgG binding antibody were detectable in all rats, at 2 weeks after administration. No IgG antibody against adenovirus vector were detectable.

5.4 Previous clinical study

Phase I clinical study

A phase I study of recombinant novel recombinant coronavirus vaccine (adenovirus vector) was launched in Wuhan in March 16, 2020. Three groups of low, medium and high dose with 36 subjects enrolled in each group were immunized with the candidate vaccine. Three doses of 5×10^{10}vp, 1×10^{11}vp or 1.5×10^{11}vp were used in the study.

Safety data collected at 28 days after vaccination showed that, the overall incidence rates of adverse reactions in low, medium and high dose groups were 83.33%, 83.33% and 75.00%, respectively. The incidence rate of grade 3 adverse reactions was 5.56%, 5.56% and 16.67% in three groups. Neither grade 4 adverse reaction nor serious adverse event was observed. The most common local adverse reaction was pain, and the most common systemic adverse reaction was fever.

The recombinant novel coronavirus vaccine (adenovirus vector) was safe in low and medium dose groups and showed a clinically tolerable safety in high dose group.

Immunogenicity data showed that, for the neutralizing antibody measured by wild-type assay: the seroconversion rate, GMT and GMI collected at 28 days after vaccination were higher than those collected at 14 days after vaccination in low, medium and high dose groups . On the 28th day, there was no statistical difference in the seroconversion rate, GMT and GMI between the low dose and the medium dose group; while, seroconversion rate, GMT and GMI between low and high dose, or between medium and high dose were statistically different. The humoral immune response induced by high dose was better than that induced by low and medium dose.

Cellular immune response after vaccination: levels of IFN-γ collected at 14 days after vaccination measured by ELISpot were obviously higher than that collected at 28 days after vaccination. There was no significant difference in the levels of IFN-γ measured by ELISpot on the 14th day after vaccination between the medium and high dose groups; the level of IFN - γ measured by ELISpot on the 14th day after vaccination in the low dose group was significantly lower than that in the medium or high dose group, with statistically difference; the levels of IL-2, IFN - γ and TNF α expressed by CD4 + and CD8 + T lymphocytes also had

similar character.

In conclusion, Ad5-nCoV vaccine was safe and immunogenic in healthy adults. The specific humoral and cellular immune responses against SARS-CoV-2 peaked at 28 days and 14days after vaccination, respectively.

Phase II clinical study

Based on the results of phase I clinical study, medium and low dose of vaccine were selected to enter phase II clinical study, which was launched in Wuhan, China in April 12, 2020. It was a randomized, double-blind and placebo-controlled study. 508 healthy adults aged 18 years and above were randomized into low dose（5×10^{10}vp）, medium dose（1×10^{11}vp） and placebo at the ratio of 2:1:1. Safety data are showed as below:

The overall incidence rates of adverse reaction collected within 7 days and 14 days after vaccination in the low, medium dose group and placebo were 74.42%, 74.70% and 37.30%, respectively. There was no statistical difference between low dose group and medium dose group. The incidence rate of adverse reaction in low and medium dose group were significantly higher than that in placebo. The incidence rate of grade 3 adverse reaction was 0.78%, 9.49% and 0% in low, medium dose group and placebo. Neither grade 4 adverse reaction nor serious adverse event was observed. The most common local adverse reactions were injection site pain, and the most common systemic adverse reactions were fatigue, headache and fever.

The recombinant novel coronavirus vaccine (adenovirus vector) was safe in low and medium dose groups, with better safety in low dose group.

Immunogenicity data showed that, the seroconversion rates of low, medium dose groups and placebo were significantly different, with seroconversion rates obviously higher in low and medium groups than placebo. The seroconversion rate of anti-S RBD binding antibody was relatively high in low and middle dose groups. On the 28th day of vaccination, the seroconversion rate of neutralizing antibody measured by pseudo-neutralization assay in low and medium dose groups was significantly higher than that in placebo.

Cellular immunity data showed that, the seroconversion rate of IFN - γ measured by ELISpot in low and medium dose groups was significantly higher than that in placebo. The recombinant

novel coronavirus vaccine (Adenoavirus vector) with low dose and medium dose showed good and similar immunogenicity in human. The recombinant novel coronavirus vaccine (adenovirus vector) was immunogenic in low and medium dose groups, with similar immunogeniciy character in both groups.

6 Study vaccine

6.1 Production process

Except for carrying different foreign genes, the recombinant novel coronavirus vaccine (adenovirus vector) and approved Ad5-EBOV Ebola Vaccine (Adenovirus vector), have same biological characteristics and produced by same cell lines, culture medium and purification methods. Therefore based on the existing platform technology of adenovirus vector vaccine and production process of Ad5-EBOV Ebola vaccine.

6.2 Formulation and dosage form

The candidate vaccine is a replication-deficient recombinant adenovirus (Ad5-nCoV) expressing gene encoding spike protein of 2019-nCoV. The only difference between the candidate vaccine and recombinant Ad5-EBOV Ebola vaccine (Adenovirus vector) lies in genes encoding antigens. Refer to the formulation and production process of recombinant Ad5-EBOV Ebola vaccine (Adenovirus vector) ,the specification of candidate vaccine is 0.5ml/dose, containing 5×10^{10}vp of recombinant replication-deficient human adenovirus type 5 expressing spike protein of SARS-CoV-2.

Placebo contains same ingredients with candidate vaccine, except for replication-deficient human adenovirus type 5 expressing spike protein of SARS-CoV-2. Specification of placebo is 0.5ml/dose.

6.3 Stability study

The stability study was carried out according to the stability research plan. The preliminary results showed that the candidate vaccine could be stored for 2 weeks under the condition of 37

± 2 ℃; under the condition of 25 ± 2 ℃, up to now it has been stored for 2 months, the results met the quality standard, the stability study under this condition is ongoing; for the condition of 5 ± 3 ℃, the 3-month point detection has been completed until now, and the result met the requirements, and the stability study at 2-8 ℃ is ongoing.

6.4 Quality research and verification of vaccines

This study is based on Chinese Pharmacopoeia Volume III (2015 Edition) of viral biological products, 《technical guidelines for the prevention of live vaccine preparations using virus as carrier》, and《technical guidelines for human gene therapy research and preparation quality control》(hereinafter referred to as the guiding principles). In combination with the quality standard of “recombinant Ebola virus vaccine (adenovirus vector)” which has been approved （YBS05112019）, and the data of this project, the quality standard of recombinant novel coronavirus vaccine (adenovirus vector) harvested liquid, raw liquid, semi-finished products and finished products was formulated.

6.5 Packaging of vaccine

The vaccine will be packaged in a labeled box. The label should contain at least the following information: vaccine name, batch number and expiration date, vaccine storage conditions and wording of "for clinical research use only".

Sample: Bottle label

Immunogenicity study of two doses of recombinant novel coronavirus vaccine (adenovirus vector)

Vaccine number：XXX

Lot：　　Date of production：

Specification：0.5ml/ vial　　Valid until：

Stored and transported at：2～8℃ in dark （For clinical use only）
Institute of Biotechnology, Academy of Military Medical Sciences
CanSino Biologics Inc

Sample: Packaging box label

Immunogenicity study of two doses of recombinant novel coronavirus vaccine (adenovirus vector)

Vaccine number：XXX

Lot：　　Date of production：

Specification：0.5ml/ vial　　Valid until：

Stored and transported at：2～8℃ in dark （For clinical use only）
Institute of Biotechnology, Academy of Military Medical Sciences
CanSino Biologics Inc

6.6 Transportation and preservation

The candidate vaccine must be stored in a safe place with lock and not accessible to unauthorized persons. Before the enrollment, investigate site should be evaluated for its storage conditions to

ensure the candidate vaccine be stored appropriately.

Transportation of vaccines includes journey from Institute of Biotechnology, Academy of Military Medical Sciences to the investigate site, and return journey of rest unused vaccines. The transportation temperature should be maintained at 2-8 ℃. When the vaccine is received, the quantity, quality and cold chain maintenance must be checked, and the corresponding records shall be filled and stored.

Temperature monitoring instruments should be used to monitor the storage and transportation temperature of the candidate vaccine every day, and the temperature should be recorded every working day (once in the morning and once in the afternoon).

Once the temperature deviation occurs, e.g. the temperature exceeds the specified range of 2-8 ℃, investigator and sponsor should be informed immediately. At the same time,《Report of vaccine cold chain damage form》 should be filled in and recorded. Vaccine deviated of temperature should be placed separately, marked and suspended. Once the temperature exceeds the specified range, candidate vaccine should be used continually only approved by the Institute of Biotechnology, Academy of Military Medical Sciences with written consent. Vaccines that do not meet the requirements should be sealed on site.

7 Study objective

To evaluate the immunogenicity and safety of the novel coronavirus vaccine (adenovirus vector) in adults aged 18 and older via intramuscular, mucosal and intramuscular/mucosal routes of two doses vaccination.

8 Study design

8.1 Design method

Subjects in the study will be randomized and stratified by route of administration, vaccination schedule (A group excluded). Total 144 Subjects will be enrolled into 6 parallel groups randomly:

A group , 2 IM doses with 56 days apart (24); C group, 2 doses of IM and mucosal immune(high dose) with 28 days apart (24); D group, 2 doses of mucosal immune(high dose) with 28 days apart (24); E group, 2 doses of mucosal immune(low dose) with 28 days apart (24); F group, 1 IM dose as a contrast (24); G group, 2 IM doses in right and left arms simultaneously. Subjects will randomized into 6 groups listed above as a ratio of 1:1:1:1:1:1, stratified by sex (Male/ Female) and age (18~55 years, and ≥ 56 years).

The procedures of grouping, vaccination and sample collection are shown in the table below:

<table>
<tr><th rowspan="2">Group</th><th rowspan="2">Sample size</th><th colspan="2">Route of administration</th><th rowspan="2">Schedule</th><th rowspan="2">Procedure</th><th rowspan="2">Note</th></tr>
<tr><th>1st dose</th><th>2nd dose</th></tr>
<tr><td>A</td><td>24</td><td>IM</td><td>IM</td><td>Day0,56</td><td>Before and 28 days after 1st dose，before and 14days, 28days and 6 months days after 2nd dose</td><td>People who had been inoculated with intramuscular injection of 1 dose</td></tr>
<tr><td>C</td><td>24</td><td>IM</td><td>Mucosal vaccination</td><td>Day0,28</td><td rowspan="3">Before and 14 days after 1st dose，before and 14days, 28days and 6 months days after 2nd dose</td><td>High dose for mucosal vaccination</td></tr>
<tr><td>D</td><td>24</td><td>Mucosal vaccination</td><td>Mucosal vaccination</td><td>Day0,28</td><td>High dose</td></tr>
<tr><td>E</td><td>24</td><td>Mucosal vaccination</td><td>Mucosal vaccination</td><td>Day0,28</td><td>Low dose</td></tr>
<tr><td>F</td><td>24</td><td>IM</td><td>--</td><td>Day0</td><td>Before and 14 days, 28 days, 6 months after vaccination</td><td>--</td></tr>
<tr><td>G</td><td>24</td><td>IM</td><td>IM</td><td>Day0</td><td>Before and 14 days, 28 days, 6 months after vaccination</td><td>Left,right arm for each dose</td></tr>
<tr><td>Total</td><td>144</td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

8.2 study endpoint

8.2.1 Primary endpoint

1. Safety endpoint

Incidence of adverse events (AE) for 0-7 days post each vaccination

2. Immunogenicity endpoints

Geometric Mean Concentration (GMC) and seroconversion rates of SARS-CoV-2 Spike RBD-specific antibody levels (ELISA) 28 days post full vaccination

Geometric Mean Titre (GMT) and seroconversion rates of SARS-CoV-2 neutralizing antibody levels 28 days post full vaccination

8.2.2 Secondary endpoint

1. Safety endpoint

Incidence of adverse events (AE) for 30/60 minutes (30 mins for IM, 60 mins for mucosal) post vaccination;

Incidence of adverse events (AE) for 8-28 days post each vaccination;

Incidence of serious adverse events (SAE) occurring from 1st dose until 6 months post full vaccination.

2. **Immunogenicity endpoint**

①GMC and seroconversion rates of SARS-CoV-2 Spike RBD-specific antibody levels (ELISA) detected before 1st dose of all groups, before 2nd dose(F, G excluded), 14 days post 1st or 2nd dose;

②GMT and seroconversion rate of SARS-CoV-2 neutralizing antibody detected before 1st dose of all groups, before 2nd dose(F, G excluded), 14 days post 1st or 2nd dose;

③IFN-γ level (ELISpot) detected before 1st dose of all groups,14 days post vaccincation (A excluded), before (F ,G excluded) and 14 days post 2nd dose.

④ SARS-CoV-2 neutralizing antibody against AdHu5 detected before 1st dose of all groups, before 2nd dose(F, G excluded), 14 days and 28 days post 2nd dose;

⑤GMI of SARS-CoV-2 Spike RBD-specific antibody (ELISA) detected before 1st dose of all groups, before 2nd dose(F, G excluded), 14 days and 28 days post 2nd dose;

⑥For A group: GMC, seroconversion rate and GMI of SARS-CoV-2 Spike RBD-specific antibody (ELISA) detected 28 days post 1st dose; GMT and seroconversion rate of SARS-CoV-2 neutralizing antibody (pseudovirus) post 1st dose; SARS-CoV-2 neutralizing antibody against AdHu5.

8.2.3. Exploratory endpoints

① GMC of SARS-CoV-2 Spike RBD-specific antibody (ELISA), SARS-CoV-2 Spike-specific antibody (ELISA) and GMT of neutralizing antibody against AdHu5 detected 6 months post full vaccination

② IgA antibody levels detected before 1st dose of all groups (A, G excluded), 14 days post 1st dose, 14 days and 28 days post 2nd dose;

③ T cell specific cytokines and other mucosal immune-related characteristics detected by flow cytometry.

8.3 study procedure

Visiting plan

Group A:

8 visits, including V0 (1st vaccination day), V1(28 +3 days post 1st dose), screening visit (-3 days to the 2nd vaccination day), V2 (56+5 days post 1st dose / 2nd vaccination day), V3 (8+1 days post 2nd dose), V4 (14+1 days post 2nd dose), V5 (28+3 days post 2nd dose), V6 (6 months± 15 days post full vaccination).

① V0 (1st vaccination day): informed consent form signed, blood collection at baseline, vaccination, observation after vaccination;

② V1 (28 +3 days post 1st dose): observation of adverse events, blood collection;

③ Screening visit (-3 days to the 2nd vaccination day): Confirm written informed consent has been obtained, screening, check inclusion and exclusion criteria, exclusion of ineligible subjects;

④ V2 (56+5 days post 1st dose / 2nd vaccination day): assignment of study number, blood collection, vaccination, observation after vaccination, issue Diary card;

⑤ V3 (8+1 days post 2nd dose): observation of adverse events, collection of Diary card, issue contact card;

⑥ V4 (14+1 days post 2nd dose): blood collection, observation of serious adverse events;

⑦ V5 (28+3 days post 2nd dose): blood collection, observation of adverse events, and collection of contact card;

⑧ V6 (6 months± 15 days post full vaccination): collection of blood, observation of serious adverse events.

Group C/D/E:

9 visits, including screening visit (-3 day to -1 day before V0), V0 (1st vaccination day), V1(8 +1 days post 1st dose), V2 (14+2days post 1st dose), V3 (28+3 days post 1st dose / 2nd vaccination day), V4 (8+1 days post 2nd dose), V5 (14+2 days post 2nd dose), V6(28+3 days post 2nd dose), V7(6 months± 15 days post full vaccination).

① Screening visit (-3 day to -1 day before V0): Confirm written informed consent has been obtained, screening, check inclusion and exclusion criteria;

② V0 (1st vaccination day): randomization, blood collection at baseline, collection of

nasopharyngeal swabs, vaccination, observation after vaccination, issue Diary card (Day 0-7);

③ V1 (8 +1 days post 1st dose): laboratory test for safety, observation of adverse events, collection of Diary card, issue Contact card;

④ V2 (14+2 days post 1st dose): blood collection, collection of nasopharyngeal swabs, observation of serious adverse events;

⑤ V3 (28+3 days post 2nd dose/ 2nd vaccination day): collection of Contact card, blood collection, collection of nasopharyngeal swabs, laboratory test for safety, vaccination, observation after vaccination, issue Diary card;

⑥ V4 (8+1 days post 2nd dose): laboratory test for safety, observation of adverse events, collection of Diary card, issue Contact card;

⑦ V5 (14+2days post 2nd dose): collection of blood and nasopharyngeal swabs, observation of serious adverse events;

⑧ V6(28+3 days post 2nd dose): collection of blood and nasopharyngeal swabs, observation of adverse events, collection of Contact card (Day 8-28);

⑨ V7 (6 months± 15 days post full vaccination): collection of blood, observation of serious adverse events;

Group F/G:

6 visits, including screening visit (-3 day to -1 day before V0), V0 (1st vaccination day), V1(8 +1 days post 1st dose), V2 (14+2days post 1st dose), V3 (28+3 days post 1st dose), V4(6 months± 15 days post full vaccination).

① Screening visit (-3 day to -1 day before V0): Confirm written informed consent has been obtained, screening, check inclusion and exclusion criteria;

② V0 (1st vaccination day): randomization, blood collection at baseline, collection of nasopharyngeal swabs (G excluded), vaccination, observation after vaccination, issue Diary card;

③ V1 (8 +1 days post 1st dose): laboratory test for safety, observation of adverse events, collection of Diary card, issue Contact card;

④ V2 (14+2 days post 1st dose): blood collection, collection of nasopharyngeal swabs (G excluded), observation of serious adverse events;

⑤ V3 (28+3 days post 2nd dose/ 2nd vaccination day): blood collection, collection of nasopharyngeal swabs (G excluded), observation of adverse events, collection of Contact card;
⑥ V4 (6 months± 15 days post full vaccination): collection of blood, observation of serious adverse events.

Table 8-3-1-1 Schedule of Activities – Group A

Visit number	***Screening**	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Scheduled Day	-3-0 day of V2	Dose 1	28 days after Dose 1	56 days after Dose 1/Dose 2	8 days after Dose 2	14 days after Dose 2	28 days after Dose 2	6 months after
Time window	--	--	+3 days	+5 days	+1 day	+1 day	+3 days	±15 days
Informed consent	●	●						
Demographics collection	●	●						
HIV antibody test	●							●
Collect 2019 novel coronavirus epidemiology	●	●						
Hight, wight, blood pressure examination	●							
Axillary temperature examination	●	●		●				
Pregnancy Test (For Women of Child Bearing Potential only)	●							●
Medical History Collection	●							
Review Inclusion/Exclusion Criteria	●							
Assign study number				●				
Sample Collection		●	●	●		●	●	●
Vaccination		●		●				

30 mins post-vaccination observation		●		●				
Safety collection visit (AR/AE)		●	●	●	●		●	●
SAE collection		●	●	●	●	●	●	●
Distribute Diary Card				●				
Collect Diary Card and distribute Contact					●			
Collect Contact Card							●	
Record Vaccination and Visit	●			●	●	●	●	●
Record Medications/Non-study vaccinations		●	●	●	●	●	●	●

Table 8-3-1-2 Schedule of Blood Collection – Group A

Visit number	**Screening**	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Scheduled Day	-3-0 day of V2	Dose 1	28 days after Dose 1	56 days after Dose 1/Dose 2	8 days after Dose 2	14 days after Dose 2	28 days after Dose 2	6 months after Dose 2
Ttime window	--	--	+3 days	+5 days	+1 day	+1 day	+3 days	±15 days
HIV antibody test，HCG test	3mL	--	--	--	--	--	--	3mL
Humoral immunity(Procoagulant)	--	10mL	10mL	10ml	--	10ml	10ml	10ml
Cellular immunity(Anticoagulant)	--	--	--	20mL	--	20ml	--	--
Total blood collection	3mL	10ml	10mL	30mL	--	30ml	10ml	13mL

Table 8-3-1-3 Schedule of Activities–Other Groups (Except for Group A)

Visit number	**Screening**	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7***
Scheduled Day	-3-0 day	Dose 1	8 days after Dose 1	14 days after Dose 1	28 days after Dose 1/Dose 2	8 days after Dose 2	14 days after Dose 2	28 days after Dose 2	6 months after the last Dose
Time window	--	--	+1 day	+2 days	+3 days	+1 day	+2 days	+3 days	±15 days
Informed Consent	●								
Demographics collection	●								
HIV antibody test	●								●
2019 novel coronavirus antibody test	●								
Collect 2019 novel coronavirus	●								
Hight, wight, blood pressure	●								
Axillary temperature	●	●			●				
Pregnancy Test (For Women of Child	●								●
Blood Test	●		●		●#	●#			
Blood biochemistry	●		●		●#	●#			
Collect Medical	●								
Review Inclusion/Exclusion	●				●#				
Assign study number		●							

Blood Collection		●		●	●		●#	●#	●
Throat Swab(Except		●		●	●		●#	●#	
Vaccination		●			●#				
## Post-vaccination		●			●#				
Safety collection visit (AR/AE)		●	●		●	●#		●#	●
SAE collection		●	●	●	●	●#	●#	●#	●
Distribute Diary Card		●			●#				
Collect Diary Card and			●			●#			
Collect Contact Card					●			●#	
Record Vaccination	●	●	●	●	●	●#	●#	●#	●
Record Medications/ Non-study		●	●		●	●#		●#	●

Note: *V4 is the 6 months post the last vaccination for group F/G.

#Except for group F/G

##For post-vaccination observation, 60 mins are required for mucosal immunization group, and 30 mins are required for intramuscular injection group.

Table 8-3-1-4 Schedule of Blood Collection (Except for Group A)

Visit	Screening	V0	V1	V2	V3	V4	V5	V6	V7
Scheduled Day	Day -3-0	Dose 1	8 days after Dose 1	14 days after Dose 1	28 days after Dose 1/Dose 2	8 days after Dose 2	14 days after Dose 2	28 days after Dose 2	6 months after the last Dose
time window	--	--	+1 day	+2 days	+3 days	+1 day	+2 days	+3 days	±15 days
2019 novel coronavirus antibody test (Procoagulant)	2ml	——	——	——	——	——	——	——	——
HIV antibody test, HCG test (Procoagulant)	3ml	——	——	——	——	——	——	——	3ml
Blood Test(Anticoagulant)	2ml	——	2ml	——	2ml*	2ml*	——	——	——
Blood biochemistry (Procoagulant	3ml	——	3mL	——	3mL*	3mL*	——	——	
Humoral Immunity (Procoagulant)	——	10ml	——	10mL	10ml	——	10ml*	10ml*	10ml
Cellular Immunity(Anticoagulant)	——	20ml	——	20ml	20mL	——	20ml*	——	——
Total volume	10mL	30ml	5mL	30mL	35mL	5mL	30ml	10ml	13ml

*No blood collection for group F & G.

Group A: 6 times of humoral immunity detection against 2019 novel coronavirus were performed: before the first dose of vaccination, 28 days after dose 1; before the second dose of vaccination, 14 days after dose 2; 28 days after dose 2; 6th month after full vaccination. Two times of detection of cellular immunity against 2019 novel coronavirus were performed: before the 2nd dose vaccination, 14 days after dose 2, total≈106mL blood sample were collected.

Group F / G: two laboratory tests were performed: before vaccination and 8 days after vaccination; 4 times of humoral immunity detection against 2019 novel coronavirus were performed: before vaccination, 14 /28 days and 6 months after vaccination; 3 times of cellular immunity detection against 2019 novel coronavirus were performed: before vaccination, 14 days and 28 days after vaccination, total ≈118mL blood sample were collected.

Group C / D / E: four laboratory tests were performed: before and 8 days after each dose of vaccination; 6 times detection of humoral immunity against 2019 novel coronavirus: before and 14

days after Dose 1, before and 14 days after Dose 2, 28 days after Dose 2 and 6 months after full vaccination; 4 times detection of cellular immunity against 2019 novel coronavirus were planned: before and 14 days after each vaccination; total ≈168mL blood sample were collected.

8.4 Sample size

Subjects in the study will be randomized and stratified by route of administration, vaccination schedule (A group excluded). Total 144 Subjects will be enrolled into 6 parallel groups randomly: A group, 2 IM doses with 56 days apart (24); C group, 2 doses of IM and mucosal vaccination (high dose) with 28 days apart (24); D group, 2 doses of mucosal vaccination (high dose) with 28 days apart (24); E group, 2 doses of mucosal vaccination (low dose) with 28 days apart (24); F group, 1 IM dose as a contrast (24); G group, 2 IM doses in right and left arms simultaneously.

8.5 Study suspension and early termination

Adverse events after vaccination will be collected and reported to DSMB every week (All SAE should be reported immediately. All safety data will be reviewed by DSMB independently based on weekly report. Sponsor, principle investigator, ERCs/IRBs or regulatory authority have right to suspend or terminate the study if violations of the protocol, GCP requirements or ethical requirements happens. Reasons should be explained to other parties and Subject.

If one of the following situations occurs, the sponsor will convene an expert panel meeting involving investigators and DSMB, to determine whether to early terminate the clinical study:

① Any subject experience a Grade 4 adverse event that is considered related to vaccination by investigators;

② Any subject experience an SUSAR;

③ Occurrence of grade ≥3 adverse events that lasts for at least 48 hours in more than 20% of subjects.

④ The study has a large potential safety risk assessed by DSMB;

The study will be terminated if one of the following situations occurs:

① Sponsor decides to discontinue development of the study vaccine and explains the reason;

② ERCs/IRBs decides to discontinue development of the study vaccine and explains the reason;

③ Regulatory authority decides to discontinue development of the study vaccine and explains the reason.

8.6 Study duration

Subjects will be followed for about 8 months from enrollment to last visit. Some subjects may

terminate the study in advance.

9 study population

9.1 Selection of population

Adults aged 18 and older will be selected as the targeted population. Subjects should be informed with informed consent form approved by the ethics committee, and sign the forms as volunteer. Only meet the inclusion / exclusion criteria, they could participate in this study only after passing the physical examination and meet the following inclusion and exclusion criteria. The investigators, relevant investigators and any employees in the CRO who Subjects in this study were not allowed to be the subjects.

9.2 Inclusion criteria

Adults aged 18 years and older;
Be capable of signing the informed consent forms;
Be able and willing to comply with study protocol and complete the follow-up;
Have negative result for HIV screening;
Axillary temperature ≤ 37.0℃;
Have negative result for SARS-CoV-2 specific antibodies (IgG and IgM) detection (A group excluded);

Eligible for the study after obtaining details and results of medical history, physical examination and laboratory test.

9.3 Exclusion criteria

Exclusion criteria of 1st dose

Individual with abnormal laboratory test, or with clinically significant abnormalities judged by investigators (including, WBC count, lymphocyte count, neutrophils, platelets, hemoglobin, alanine aminotransferase ALT, aspartate aminotransferase AST, total bilirubin, fasting glucose, creatinine). (A group excluded);

Individual with oral ulcer, swollen throat and other oral diseases;

Individual with upper respiratory tract infection;

Medical history or family history of convulsions, epilepsy, encephalopathy and psychosis;

History of allergy to any components of the vaccine or serious allergic reaction;

Individual with acute febrile diseases and infectious diseases;

History of laboratory-confirmed SARS-CoV-2 infection;

Previous treatments for curing COVID-19 or vaccination of COVID-19 vaccines;

Severe cardiovascular diseases, e.g, arrhythmia, conduction block, myocardial infarction, severe hypertension and uncontrollable hypertension with medication (SBP ≥ 140mmHg, DBP≥ 90mmHg);

Serious chronic disease or in a progressive condition that cannot be controlled well, such as asthma, diabetes, thyroid disease, etc;

Congenital or acquired angioedema/ neuroedema;

Have urticarial in the past 1 year;

Congenital or functional absence of spleen;

Have thrombocytopenia or other blood coagulation disorders (Intramuscular injection is not allowed);

Individual with fainting during acupuncture treatment (in IM groups);

Receipt of immunosuppressant therapy, anti-allergic therapy, cytotoxic therapy, inhaled corticosteroid aerosol in the past 6 months (Surface corticosteroid therapy for acute non-complicated dermatitis excluded);

Receipt of blood product within 4 months prior to administration of study vaccine;

Receipt of other study drugs within 1 month prior to administration of study vaccine;

Receipt of live attenuated vaccines within 1 month prior to administration of study vaccine;

Receipt of subunit vaccines or inactivated vaccines within 14 days prior to administration of study vaccine;

Current treatment of anti-TB;

Women with positive pregnancy test, lactating women, or have plan to be pregnant within 6 months;

Ineligible for the study based on the assessment of the Investigators, including protocol deviation, or unavailable for signing the informed consent form.

Exclusion criteria of 2nd immunization:

Severe allergic reactions occurring after 1st vaccination;

Severe adverse events related to vaccination occurring after 1st vaccination;

New found failure of inclusion/ exclusion criteria, decided by investigator whether to be in the study;

Ineligible for the study based on the assessment of the Investigators.

Exclusion criteria for A group

Subjects in A group who have received 1st vaccination, completed medical history screening and

lab tests, will be excluded if not meeting inclusion / exclusion criteria judged by investigator.

9.4 Withdrawal from the study

Subjects can discontinue the study at any time during the study period, and the investigator should record and perform according to the following situations:

- Lose follow-up, early termination of the study;

- Subject withdraws consent without any reason;

- Subject requests to withdraw for reasons unrelated to the study, such as long-term going out, moving, etc., the specific reasons for withdrawal should be recorded;

- Subject requests to withdraw due to study related reasons, such as intolerable adverse reactions, intolerable biological specimen collection, the specific reasons for withdrawal should be recorded. Investigator should follow up the Subject who withdraws due to AE / SAE until the event is resolved;

- Subjects have right to terminate the study completely, including stopping vaccination, biological specimen collection and safety observation, or any other activities related to the study The clinical data of Subjects before withdrawal can be used for analysis. If the Subject forbids the investigator to continue to use all the clinical data related to himself/ herself, all the clinical data before the withdrawal will not be used for analysis;

-Subjects who has infection of the 2019 novel coronavirus within 28 days after vaccination was not included in the immunogenicity analysis. However, all the 2019 new coronavirus infections occurred from the first dose vaccination to 6 months after the second dose should be analyzed according to the requirements of SAE, especially to analyze whether there is ADE phenomenon.

Subjects can also partially withdraw from the study, such as only stop vaccination, or only stop biological specimen collection, and other study specified in the protocol should continue to be completed.

9.5 Completion of the study

9.5.1 Completion of safety observation

The safety observation is performed within 28 days after the full vaccination for the Subjects who completed the vaccination according to the protocol, and the SAE report should be collected during the whole study period.

9.5.2 Completion of the immunogenicity study

Subjects meeting the inclusion/ exclusion criteria, is vaccinated, and completes the follow-up blood collection according to the protocol.

9.6 Definition of protocol violation & protocol deviation and solutions

9.6.1 Protocol violations (including but not limited to)

- Informed consent was not performed to the subject;
- Subject is enrolled without meeting the inclusion criteria /with meeting exclusion criteria;
- The investigator improperly asked the subjects to discontinue from the study;
- Subject received the wrong research intervention (mistakenly vaccinated with other groups of vaccine);
- Subject received vaccine that did not meet the requirements;
- Any other reason considered by the investigator and confirmed by the primary investigator.

In case of protocol violation occurred, the investigator shall report to the principle investigator and the sponsor in time, and the principle investigator should give opinions on how to deal with the incident, and pay special attention to the subject involved, conduct safety follow-up and collect safety information to ensure the safety of the Subject.

The investigator / monitor should report the protocol violation to the principle investigator, project coordinator and ethics committee by fax / email as soon as possible.

9.6.2 Protocol Deviation

- Exceeding the visit scheduling window;

- Poor compliance with the Subject, and the Subject cannot complete the blood sample collection;

- Serious adverse events (SAE) is not reported in a timely manner.

-Subject is treated with prohibited medications (intramuscular, oral or intravenous systemic corticosteroids ≥ 2 mg/kg/day, continuous use for more than 14 days, or other immunosuppressants;

- Insufficient interval between candidate vaccine and other vaccines;

- Other reasons considered by the investigator and were confirmed by the principle investigator.

The events of protocol deviation should be recorded in detail. For the subject who exceed the scheduling window or have insufficient interval between candidate vaccine and other vaccines, data will continue to be included in the safety analysis and immunogenicity analysis; for the other Subjects who deviate from the protocol, their data will be included in the safety analysis but not the immunogenicity analysis. Special cases can be included in the analysis after discussion by the safety data monitoring committee.

9.7 Collection of pregnancy related events

Pregnancy related events within 6 months after the first dose of vaccination will be collected by the investigator. The pregnancy related event report form should be filled in once knowing that the Subject is pregnant. The event should be reported to: (1) PI (2) sponsor (3) monitor by telephone, fax or email within 5 calendar days. All pregnant women in the collection period of pregnancy related events should be followed up to the end of pregnancy, and the outcomes should be recorded, including pregnancy outcome, delivery characteristics (duration of pregnancy, outcome, delivery), and newborn baby will be observed from birth to one month after birth (gender, weight, height, neonatal score). In this study, pregnancy is not considered as a serious adverse event, but should be managed according to SAE requirements. The investigator shall fill in the pregnancy event form within 24 hours after knowing the pregnancy event occurred during the study, and report it to the sponsor / CRO entrusted by the sponsor; any complications during pregnancy shall be recorded as adverse events, if any of those meet the criteria of serious adverse events, they need to be recorded as serious adverse events, such as spontaneous abortion, dead fetus, stillbirth and congenital abnormalities of infants, they need to be filled in the report form of serious adverse events within 24 hours and reported to the sponsor / CRO entrusted by the sponsor.

10. Methods and procedures

10.1 Subject screening

10.1.1 Pre-enrollment screening

The target population is adults aged 18 and older, and the recruitment advertisements approved by the ethics committee is used for recruitment publicity; The volunteer will be screened prior to enrollment after signing the informed consent form approved by the ethics committee. Before signing the informed consent form, the investigator should ensure that the volunteers have enough time to consider whether to participate in the study, and the volunteers have the opportunity to ask about the details of the study and get detailed answers. During the study, the Subjects have right to withdraw from the study at any time.

Before enrollment, volunteers will be screened and collected the following information:

-Demographics data

-Physical examination

-Height, weight, blood pressure

-Medical history

-Inclusion criteria met but not exclusion criteria.

Base on the above physical examination and interrogation screening content, the investigator judges whether the volunteer is qualified or not.

10.1.2 Pre-enrollment screening

10.1.2.1 HIV antibody screening

Perform HIV antibody test, volunteers of antibody negative can be enrolled. Volunteers with negative HIV antibody test will be enrolled into the study.

10.1.2.2 SARS-CoV-2 antibody screening

Volunteers will be detected with SARS-CoV-2 IgM and IgG antibodies. Anyone with positive results will be excluded from the study.

10.1.2.3 Blood pregnancy test

In this study, women of childbearing age in the target population will be tested for HCG during the screening period, and only the volunteer with negative results could be enrolled.

10.2 Enrollment

The subject will be given the screening number according to the order of participation in the screening. The format of screening number is Sxxx, where XXX is a three digit number, representing the sequence of screening.

In order to control the selective bias of age and gender, the following age and gender ratios are used for enrollment, details as shown in table 10-2-1. The investigator will assign the study number (for Group C-G study number is randomization number) according to the sequence of the subjects who passed the screening to the study number allocation link, and will enter the group according to the age and gender according to the table below, and fill the screening number and initials in the corresponding column of the random allocation table, and the corresponding number is the subject's study number. The study number is formed with 1 stratified number (1 for male, aged 18-55 years old; 2 for male, aged over 56 years old; 3 for female, aged 18-55 years old; 4 for female, aged over 56 years old). +1 group number(group A: 2 doses of intramuscular - interval immunization group; group C: intramuscular / mucosal - high dose interval immunization group; group D: 2 doses of mucosal - high dose interval immunization group; Group E: 2 doses of mucosal - low dose interval immunization group; Group F: 1 dose of intramuscular injection control group; Group G: 2 doses of intramuscular injection left- right arm simultaneous immunization group) + 2 digits of sequence number (01, 02, 03,...stands for the enrollment sequence of each stratification and group) It is composed of 4 digits.

Table 10-2-1 Matching of Subject gender, age and study number

Gender	Age	Study number range	Back-up study number range
Male	Aged 18-55	1A01-1A09, 1C01-1C09,1D01-1D09, 1E01-1E09,1F01-1F09， 1G01-1G09	1A10-1A11, 1C10-1C11,1D10-1D11, 1E10-1E11,1F10-1F11， 1G10-1G11

Male	Aged ≥56	2A01-2A03, 2C01-2C03,2D01-2D03, 2E01-2E03,2F01-2F03， 2G01-2G03	2A04-2A05, 2C04-2C05,2D04-2D05, 2E04-2E05,2F04-2F05， 2G04-2G05
Female	Aged18-55	3A01-3A09, 3C01-3C09,3D01-3D09, 3E01-3E09,3F01-3F09， 3G01-3G09	3A10-3A11, 3C10-3C11,3D10-3D11, 3E10-3E11,3F10-3F11， 3G10-3G11
Female	Aged ≥56	4A01-4A03, 4C01-4C03,4D01-4D03, 4E01-4E03,4F01-4F03， 4G01-4G03	4A04-4A05, 4C04-4C05,4D04-4D05, 4E04-4E05,4F04-4F05， 4G04-4G05

Due to the special epidemic situation, this study is an open study, and the vaccine is not blinded in advance. In addition to the candidate vaccines used in the study, the sponsor should also provide 40 candidate vaccines for back-up. Vaccines should be discarded if they are damaged or unusable. Although it is not necessary to inform the sponsor immediately in such cases (except for cold chain accidents), the investigator should make a detailed record of the damage of candidate vaccine.

10.3 Vaccination

10.3.1 Candidate vaccine

The candidate vaccine in this study is jointly developed by Institute of Biotechnology, Academy of Military Medical Sciences and CanSino Biologics Inc.

The vaccine is a recombinant novel coronavirus vaccine (adenovirus vector), liquid dosage form, the replication-deficient human adenovirus type 5 was used as a vector. It could express the specific S protein of SARS-CoV-2, and was produced via amplification and purification. The quality of the vaccine is in conformity with the requirements of the 《Manufacturing and verification regulation(draft) of recombinant novel coronavirus vaccine (adenovirus vector) 》, and was certified by the China Institute for Food and Drug Control.

In addition to providing sufficient candidate vaccines, the sponsor should also provide vaccines for back-up according to the requirements of the protocol. If the vaccine is damaged or cannot be used, it should be discarded. Although it is not necessary to inform the sponsor immediately

(except for cold chain accidents), but the investigator should make a detailed record of the damage of the vaccine.

10.3.2 Vaccination procedure, dosage and route of administration

The procedure, dosage and route of administration of vaccination for the six groups in this study are shown in the table

Table 10-3-2-1 Vaccination procedure and route table

Group	Route of Administration		Vaccination procedure	Dosage	Note
	1st dose	2nd dose			
A	Intramuscular injection	Intramuscular injection	Day 0, 56	Intramuscular injection 5×10^{10} vp (0.5ml)	Recruit subjects who have received one dose of the vaccine intramuscularly
C	Intramuscular injection	Mucosal vaccination	Day 0, 28	Intramuscular injection 5×10^{10} vp (0.5ml); Mucosalimmunity 2×10^{10}vp (0.2ml)	High dose for the mucosal vaccination.
D	Mucosal vaccination	Mucosal vaccination	Day 0, 28	Mucosal vaccination 2×10^{10} vp (0.2ml)	High dose
E	Mucosal vaccination	Mucosal vaccination	Day 0, 28	Mucosal vaccination 1×10^{10} vp (0.1ml)	Low dose
F	Intramuscular injection	--	Day 0	Intramuscular injection 5×10^{10} vp (0.5ml)	--
G	Intramuscular injection	Intramuscular injection	Day 0	Intramuscular injection 5×10^{10} vp(0.5ml) 2 vaccine	One dose for left and right arm separately.

10.3.2.1 Intramuscular Injection

The injection site was disinfected with 75% alcohol before injection, and the candidate vaccine was injected intramuscularly after the skin was slightly dry. The vaccine should be fully shaken before use. The vaccine cannot be injected intravenously, intradermally or subcutaneously. After vaccination, the Subjects should be carefully observed for at least 30 minutes, and appropriate emergency medical treatment should be prepared to deal with the possible allergic reaction after vaccination.

The vaccination dose: 5×10^{10}vp / 0.5ml/syringe.

10.3.2.2 Mucosal vaccination

The special device for mucosal vaccination was used as below procedure:

(1) Connect the device to the suction nozzle and make sure that the medicine cup and suction nozzle face up.

(2) Inject the test vaccine from the pre sealed syringe into the sterile penicillin bottle, extract 0.2ml test vaccine (high dose group) or 0.1ml test vaccine (low dose group) with pipette, and inject the vaccine from the pipette into the bottom of medicine cup.

(3) After the subject clamp their noses and take a deep breath, mouth with suction, and starts the instrument and immunization. Inhale slowly until can't inhale, hold the breath for 3 seconds, and move the suction nozzle away; after exhalation, put the suction mouth in your mouth and inhale again. Repeat this breathing for many times until there is no residual in the medicine cup, and the immunization is over.

(4) After immunization, press the power on / off button and the indicator light goes out.

Notes for attention

(1) Vaccine should be added into the bottom of the cup to avoid splashing on the wall of the cup, and the pipette head should not touch the bottom of the cup.

the subjects are required to clamp their noses during immunization

(2) After vaccination, the Subjects are required to fast and deprive water for half an hour.

(3) The vaccination equipment can be reused, and the suction nozzle can only be used for a single time. The vaccination equipment will be handed over to investigator at the end of the day of immunization.

(4) After mucosal immunization, the subject has to stay for 60 minutes for observation.

10.3.3 Management of vaccine

The sponsor should provide sufficient candidate vaccines, including backup vaccines. The packaging of vaccine must meet the requirements of clinical study. The sponsor is responsible for transporting the vaccine to the clinical study site, submitting the transport temperature record of the vaccine (consistent with the cold chain temperature of the vaccine) and the inspection report (qualified). The vaccine management personnel of the research institution and the vaccine management personnel of the investigate site shall jointly check the vaccine and sign the documents with the sponsor.

The cold storage in the clinical investigate site will be provided with a defined person, a defined area and a lock to store the vaccines. Unauthorized persons are not allowed to contact the vaccine. It is strictly forbidden to inject the vaccine to persons other than the subjects.

The cold storage should be equipped with temperature recorder to monitor the real-time temperature. The cold storage manager should inspect cold storage in the morning and afternoon every day and record the temperature to ensure cold storage work well. If the temperature of cold storage exceeds the range of cold chain temperature, it should be adjusted or repaired in time. The candidate vaccines should be temporarily sealed, and the written report should be submitted to the sponsor in time. The vaccines can only be used after obtaining the written approval of the sponsor. Vaccines that do not meet the requirements should be sealed on site, and no further use is allowed.

During the study, the candidate vaccine should be stored in refrigerator or freezer, equipped with thermometer. The vaccine administrator should record the temperature once an hour.

The vaccine administrator will distribute vaccines to vaccination staff according to study number of Subjects. After the vaccination, packaging of vaccines should be recovered, Distribution and left packaging should be recorded in detail.

After completion of the vaccination day, the vaccine administrator should check the number of remaining vaccines and packaging of used vaccines, all of which should be gathered and put into storage. The inner packaging (pre-filled syringe) should be destroyed as medical waste on site.

At the end of the study, the investigator should check all the remaining vaccines and the outer packaging and send them back to the sponsor.

During the whole study, the total number of used, unused and damaged vaccines must be consistent with the number provided by the sponsor. If there is any discrepancy, the investigator must give a statement to explain the reason.

10.3.4 Combination medication/vaccine

If the Subject has medical events during the study, the corresponding treatment and medical treatment are allowed. The medications used or the medical treatment should be recorded in time.

Subjects are not recommended to receive other vaccines during the study period, except those who need emergency vaccination due to emergency events, including rabies vaccine, tetanus vaccine, or other vaccines that need emergency vaccination. Any other vaccines administered to Subjects during the study period should be recorded in detail and belonged to protocol violation.

10.4 Safety observation

10.4.1 Safety observation methods

The safety observation during the study is as following:

(1) All Subjects should be observed for safety at the vaccination site after vaccination, with 30 minutes observation for intramuscular injection and 60 minutes observation for mucosal immunization.

(2) Within 0 to 7 days after each vaccination, subjects are urged to complete the safety observation by themselves, and record the results in the “Diary card”. Subjects should be followed up by a specially assigned personnel to complete the safety observation and recording. In group A, there is no need to fill in the Diary card after the first dose of vaccination, and the Diary card should be provided after the second dose of vaccination.

(3) Within 8 to 28 days after each dose of vaccination, the subjects should complete the safety observation by themselves, and record the results in the “Contact card". On the 28th day, the investigator will investigate retrospectively and verify the safety observation contents with Subject during visit. In group A, "Adverse event collection form" should be filled in after first dose to record the safety observation results during 0-28 days, after the second dose, "Contact card" should be filled in to record the safety observation results during 8-28 days.

(4) Group A: abnormality of blood routine and blood biochemistry tests before and 7 days after the second dose of vaccination. Other groups: abnormality of blood routine and blood biochemistry tests before and 7 days after the first dose of vaccination.

(5) During the study period (from the first dose to about 6 months after full vaccination), serious

adverse events are collected by combination of Subjects' self-reporting and investigators' regular follow-up.

10.4.2 Safety observation content and grading standards of adverse reactions / adverse events

10.4.2.1 Definition of adverse events and serious adverse events

Adverse event (AE): an adverse medical event that occurs after a patient or clinical study Subject receives a study intervention, but does not necessarily have a causal relationship with treatment. Serious adverse events (SAE): are generally defined as: (1) causing death; (2) resulting in life-threatening, which refers to the immediate risk of death of a serious patient, and does not refer to the possibility of death in the event of serious development in the future; (3) resulting in hospitalization or prolonged hospitalization ; (4) causing permanent or significant loss of function; (5) causing teratogenesis; (6) other important medical events:

10.4.2.2 Safety observation content

(1) Adverse events at 0～7 days post each vaccination.

(2) Adverse events at 8～28 days post each vaccination.

(3) Serious adverse events occurring from the first dose to 6 months post full vaccination;

(4) Other groups (group A not included): Changes in blood routine indicators (including, white blood cell count, lymphocyte count, neutrophil, platelet, hemoglobin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, fasting blood glucose and creatinine) collected before and 7 days post vaccination will be analyzed.

10.4.2.3 Grading standards of adverse events

According to the 《Guidelines for grading standards of adverse events in clinical study of prophylactic vaccines》 (No. 102, 2019) issued by the State Drug Administration, the adverse events are graded and evaluated. See table 10-4-1-1 and table 10-4-1-2 for details.

Table 10-4-1-1 Adverse Event Grading Table after vaccination

Symptoms /Signs	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Pain, tenderness (optionally used; palpation for subjects unable to autonomously express pain)				
Pain	Does not or slightly influence limb movement	Influence on limb movement	Influence on daily life	Loss of basic self-care ability, or hospitalization
Induration *, swelling ** #	2.5 to < 5 cm in diameter or 6.25 to < 25 cm^2 in area, with no or slight influence on daily life	5 to < 10 cm in diameter or 25 to < 100 cm^2 in area or interfere with daily life	≥ 10 cm in diameter or ≥ 100 cm^2 in area or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or significant impact on daily life	Abscess, exfoliative dermatitis, necrosis of dermis or deep tissue
Rash *, blush ** #	2.5 to < 5 cm in diameter or 6.25 to < 25 cm^2 in area, with no or slight influence on daily life	5 to < 10 cm in diameter or 25 to < 100 cm^2 in area or interfere with daily life	≥ 10 cm in diameter or ≥ 100 cm^2 in area or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or significant impact on daily life	Abscess, exfoliative dermatitis, necrosis of dermis or deep tissue
Other				
Pruritus	Vaccination site pruritus, which resolved spontaneously or within 48 h after treatment	Vaccination site pruritus, which did not resolve within 48 h after treatment	Influence on daily life	NA
Cellulitis	NA	Need for non-injectable treatment (e.g., oral anti-bacterial, antifungal,	Need for intravenous therapy (e.g., intravenous antibacterials, antifungals, antivirals)	Sepsis, or tissue necrosis, etc.

Symptoms /Signs	Grade 1	Grade 2	Grade 3	Grade 4
		antiviral therapy)		
Diarrhea	Mild or transient, 3-4 times/day, abnormal stool appearance, or mild diarrhea lasting less than 1 week	Moderate or persistent, 5-7 times/day, abnormal stool appearance, or diarrhea for > 1 week	> 7 times/day, abnormal stool appearance, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, requiring > 2 L intravenous fluids	Hypotensive shock, Need to be hospitalized
Constipation*	Requires stool softener and dietary modification	Need for laxatives	Stubborn constipation requires manual dredging or the use of enemas	Toxic megacolon or ileus
Dysphagia	Mild discomfort while swallowing	Dietary restrictions	Eating, talking very limited; unable to eat solid foods	Cannot eat liquid food; requires parenteral nutrition
Anorexia	Decreased appetite without decreased food intake	Decreased appetite, decreased food intake without significant weight loss	Decreased appetite with significant weight loss	Intervention Required (e.g. tube feeding, parenteral nutrition)
Vomiting	1-2 times/24 h without influence on activities	3-5 times/24 h or limited activities	> 6 episodes in 24 h or need for intravenous hydration	Shock due to hypotension requiring hospitalization or other routes of nutrition
Nausea	Transient (< 24 h) or intermittent and essentially normal food	Sustained nausea leading to reduced food intake (24-48 h)	Persistent nausea resulting in little to no food intake (> 48 h) or need for intravenous	Life-threatening (e.g. hypotensive shock)

Symptoms /Signs	Grade 1	Grade 2	Grade 3	Grade 4
	intake		hydration	
Musculoskeletal and connective tissue				
Myalgia (non-vaccination site)	No influence on daily activities	Slight influence on daily activities	Severe muscle pain, severe interference with daily activities	Emergency or hospitalization
Arthritis	Mild pain with inflammation, erythema, or joint swelling; does not interfere with function	Moderate pain with inflammation, erythema, or joint swelling; preventing function but not interfering with daily activities	Severe pain with inflammation, erythema, or joint swelling; interference with daily activities	Permanent and/or disabling joint damage
Arthralgia	Mild pain, no interference with function	Moderate pain; pain requiring analgesics and/or pain preventing function but not influencing on daily activities	Severe pain; requiring analgesics and/or pain influencing on daily activities	Disabling pain
Nervous System				
Headache	No influence on daily activities, no treatment required	Transient, slight influence on daily activities, may require treatment or intervention	Significant disruption of daily activity requiring treatment or intervention	Intractable requiring emergency room visit or hospitalization
Syncope	Close to syncope without loss of consciousness (e.g. pre-syncope)	Loss of consciousness without treatment	Loss of consciousness requiring treatment or hospitalization	NA
New	NA	NA	1-3 times	Prolonged and repeated

Symptoms /Signs	Grade 1	Grade 2	Grade 3	Grade 4
convulsions			convulsions	convulsions (e.g. status convulsion) or difficult to control (e.g. intractable epilepsy)
Respiratory System				
Cough	Transient, no treatment required	Persistent cough, effective treatment	Paroxysmal cough that cannot be controlled by treatment	Emergency or hospitalization
Acute bronchospasm	Transient; no treatment required; FEV_1% 70-80%	Requires treatment; bronchodilator therapy normalized; FEV_1% 50-70%	Bronchodilator treatment does not return to normal; FEV_1% 25% to 50% or persistent depression in the intercostal space	Cyanosis; FEV_1% < 25%; or intubation required
Dyspnea	Dyspnea on exercise	Dyspnea with normal activity	Dyspnea at rest	Dyspnea requiring oxygen therapy, hospitalization or assisted breathing
Organ system symptom/sign	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Skin and subcutaneous tissue				
Non-vaccination site pruritus (no skin lesions)	Slight itching, no or slight influence on daily life	Pruritus influencing on daily life	Itching prevents daily life	NA
Mucocutaneous disorder	Erythema/Itching/Color Altered	Diffuse rash/maculopapular rash/dryness/desquamation	Herpes/oozing/desquamation/ulceration	Exfoliative dermatitis involving mucosa, erythema multiform, or suspected Stevens-Johnsons syndrome

Symptoms /Signs	Grade 1	Grade 2	Grade 3	Grade 4
Psychiatric system				
Insomnia*	Mild difficulty falling asleep, no or slight influence on daily life	Moderate difficulty falling asleep,influence on daily life	Severe difficulty falling asleep, severe influence on daily life, treatment or hospitalization required	NA
Irritation or depression	Mild irritability or mild depression	Irritability or somnolence	Unable to soothe or become hyporesponsive	NA
Mental disorder (includes anxiety, depression, mania, and insanity) Detailed symptoms to be reported	Minor symptoms that do not require medical attention or behavior do not influence or slightly influence daily life	Clinical symptoms requiring medical attention or behavior influencing on daily life	Requires hospitalization or inability to perform daily life	Have a tendency to hurt yourself or others or acute insanity or loss of basic self-care ability
Immune system				
Acute allergic reactions **	Localized urticarial (blistering) not requiring treatment	Localized urticarial requiring treatment or mild angioedema not requiring treatment	Extensive urticarial or angioedema requiring treatment or mild bronchospasm	Anaphylactic shock or life-threatening bronchospasm or laryngeal edema
Other				
Fatigue, asthenia	No influence on daily activities	Influence normal daily activities	Seriously influence daily activities and cannot work	Emergency or hospitalization
Non-vaccination site pain	Mild pain, no or slight influence on	Pain influencing on daily life	Pain incapacitating for daily life	Disabling pain, loss of basic self-care ability

Symptoms /Signs	Grade 1	Grade 2	Grade 3	Grade 4
(Identify location when reporting)	daily life			
Sore throat***	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe in severity, severe interference with daily activities, treatment required	NA
Xerostomia	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA
Hoarseness	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA
Oral mucositis	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA
Garget	Transient, no treatment required, no influence on daily activities	Slight influence on daily activities	Severe interference with daily activities, treatment required	NA

Note: * In addition to directly measuring the diameter to grade the evaluation, record the progressive change of measurement results.

** The largest measured diameter or area shall be used.

Induration and swelling, rash, and blush should be evaluated and graded based on functional class and actual measurement, and the index with the higher grade should be selected.

Note: FEV_1% refers to forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC)

* For constipation and insomnia, attention should be paid to changes before and after vaccination.

** Refers to type I hypersensitivity.

Refers to non-vaccination site pain other than myalgia, arthralgia, headache.
*** Refer to 《Guidelines for grading standards of adverse events in clinical studys of prophylactic vaccines》

Table 10-4-1-2 Vital Signs Grading Scale

Signs	Grade 1	Grade 2	Grade 3	Grade 4
Fever * [Axillary temperature (℃)]	37.3~<38.0	38.0~<38.5	38.5~<39.5	≥39.5 for more than 3 days

Note: * Axillary temperature is generally used in China. It can be converted in oral temperature and rectal temperature if necessary. Oral temperature = Axillary temperature +0.2℃; Rectal temperature = Axillary temperature ＋（0.3~0.5℃）.When persistent high fever occurs, the cause of high fever should be identified as soon as possible.

For adverse events that do not reach level 1 in the above table, it can be recorded as level 0.Among the above adverse events, pain, induration, swelling, rash, erythema, pruritus and cellulitis belong to solicited adverse events at the injection site, and the rest are collected as solicited general adverse events. Adverse events not listed above will be classified into unsolicited adverse events.

10.4.2.5 Principles of classification of other adverse events

For the adverse events that not included in the classification list above, it can be evaluated in the following grading standard.

Table 10-4-1-2 Other general principles for adverse event grading

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Mild: Short-term (< 48 h) or slight discomfort, no influence on activities, no treatment required	Moderate: mild or moderate limitation of motion, may require medical attention, no or mild treatment required	Severe: marked limitation of motion, Requires medical attention and treatment, may require hospitalization	Critical: May be life threatening, severely limited mobility, requires monitoring and treatment	Dead

10.4.3 Outcome of adverse events

The outcome of Adverse Reactions / Events includes: ① Recovered; ② Not recovered; ③ Recovered with sequelae; ④ Death; ⑤ Lost of follow-up.

10.4.4 Causal relationship between adverse events and vaccination

Investigators should try their best to judge AE and evaluate its possible causal relationship, that is, the causal relationship between vaccination and alternative causes (such as history of underlying diseases, combined treatment). This applies to all AES, including serious ones and non serious ones.

Causality assessment will be determined by the extent to which an event can be reasonably explained in one or more of the following aspects:

- Similar reactions were observed in the past;

- For similar types of preparations, the same events have been reported in the literature;

-The adverse event occurred with the vaccination of the study vaccine at the time and after the re-vaccination of the study vaccine it occurred again.

By definition, all collected AE (i.e. all collected reported local adverse events) at the vaccination site will be considered to be related to vaccination.

The causal relationship of AE should be evaluated by the investigator according to the following questions. Based on your judgment, if there is a reasonable possibility that the AE is caused by vaccination

- Definitely not related: the subject did not use the study vaccine; or the occurrence of adverse events was illogical with the time sequence of vaccination; or there were other significant reasons that could cause the adverse events.

- Probably not related: there is evidence of vaccination with the study vaccine; adverse events are more likely to be caused by other reasons, such as the clinical condition of the subject, other treatment or concomitant medication, which are inconsistent with known adverse reactions of the study vaccine; repeated vaccination of study vaccine is negative or uncertain.

-Probably related: there is evidence of vaccination of the study vaccine; the occurrence of adverse events and the time sequence of vaccination of the study vaccine are rationale; it was an adverse event or used to occurred when receiving vaccination in the past. It has casual relationship with the study vaccine. The cause of adverse events cannot be excluded by the study vaccine, however it also possibly caused by other reasons.

-Definitely related: there is an evidence of vaccination of the study vaccine; the occurrence of adverse events and the time sequence of vaccination of the study vaccine are reasonable; the explanation of occurrence of adverse events caused by the study vaccine is more reasonable than other possible reasons; repeated vaccination of study vaccine shown positive AE; the situation of the adverse events are consistent with the previous understanding of this or this

kind of vaccine, which no-doubt is the cause of the adverse event.

- Undetermined: The information is incomplete and no more supplementary information can be obtained. The relationship between adverse events and study vaccine cannot be determined.

10.4.5 Handling and reporting of serious adverse events

After vaccination with the study vaccine, there are moderate and below reactions such as dizziness, swelling, pain or (and) moderate and below fever, general discomfort, etc., which generally disappear without special treatment. In case of serious adverse events, investigator should take necessary measures promptly and report according to the requirements.

(1) SAE should be collected and recorded from vaccination to the last follow-up. If SAE occurs after the last follow-up, and the causality between SAE and vaccine cannot be excluded, the SAE still needs to be collected and recorded. In case of SAE, the investigator should fill in the 《serious adverse event report form》 as detailed as possible, sign and date it.

The investigator must report to the sponsor, medical ethics committee of Zhongnan Hospital of Wuhan University and other departments required by laws and regulations within 24 hours after learning about SAE. The contact details are as follows:

Contact person of Institute of Biotechnology, Academy of Military Medical Sciences: Hou Lihua; Tel: 010-66948565; Email: houlihua@sina.com

Contact person of medical ethics committee of Zhongnan Hospital of Wuhan University: Zheng Lei, Tel: 027-67812787; e-mail: znyysae@l26.com

Science and Education Department of Hubei Health Committee 027-87576373

(2) After receiving the SAE report, the sponsor needs to review the report and classify it. If it is SUSAR (SUSAR means suspected and unexpected serious adverse reactions whose nature and severity of clinical manifestations are beyond the suspicions and unpredictability of the existing information in such as the investigator brochure of the study drug, the instructions of the marketed drugs or the summary of product characteristics). The sponsor also needs to report to the provincial health administrative department within 7 calendar days (death or life-threatening) / 15 calendar days (other SUSAR). The first time for the sponsor to be informed

counted as day 0.

The sponsor shall immediately analyze and evaluate the safety related information from any source, including severity, causality with the study vaccine and whether it is an expected adverse event or not. The sponsor is not allowed to change the investigator's judgment on the causality between serious adverse events and vaccine at will. In case of disagreement between the sponsor and the investigator, the opinions of both the sponsor and the investigator should be explained in detail in the report and report the AE according to the higher management requirement.

(3)After receiving the relevant safety information of the clinical study provided by the sponsor, the investigator should sign after receiving and review it in time, consider the immunity and follow-up of the observed subjects, whether to make corresponding adjustment, communicate with the guardian of the observation object as soon as possible when necessary, and report the SUSAR provided by the sponsor to the ethics committee in time.

10.4.6 Record of safety observation data

Any clinically significant adverse events occurred after vaccination should be recorded in the《Diary Card》and the《Contact Card》. The investigators should investigate and verify the adverse events and conduct medical visits, such as medical history investigation, physical examination and necessary laboratory examination (if necessary). They should carry out corresponding medical treatment and continue to follow up till the end of the adverse events and complete the detailed records.

Adverse event records should include the following contents:

-Name of adverse event

-Adverse event start and stop dates

-Severity (classification)

-Causality with vaccination

-Laboratory test results

-Actions

-Outcome

In case of grade 3 or above adverse events, acute allergic reactions or SAE during the safety observation period, the investigator shall follow up until the symptoms disappeared or stabilized, and record the follow-up contents in detail on the adverse event case questionnaires.

10.4.7 Medical treatment of adverse events

If there are local or general adverse reactions / adverse events or serious adverse events after vaccination, the investigator should provide appropriate treatment or medical consultation to the subjects in time to alleviate or relieve the pain of the subjects. If necessary, the green channel of medical treatment should be started and medical treatment should be given in time. In this process, the medical treatment and results should be recorded in detail.

10.5 Collection and detection of biological samples

Calculation method of uncertainty value: when calculating GMT, GMI and positive conversion (quadruple growth) of antibodies determined by ELISA and neutralization test, if the initial dilution is negative, it shall be calculated by half of the initial value; if it is greater than the maximum dilution, it shall be calculated by the maximum dilution.

10.5.1 Detection of anti-S RBD binding antibody by ELISA

10.5.1.1 Detection time point

Before the first dose of vaccination in each group, 28 days after the first dose of vaccination (group A), before the second dose of vaccination, 14 days after the second dose of vaccination, 28 days and the 6 months after full vaccination, the levels of anti-S RBD binding antibody are detected.

10.5.1.2 Assessment

The level of anti-S RBD binding antibody 28 days after full vaccination is assessed as primary

endpoint in immunogenicity. The difference of antibody level between the groups and the change of antibody level at each time point before and after vaccination are compared.

10.5.2 Detection of Neutralizing antibody

10.5.2.1 Detection time point

Before the first dose of vaccination in each group, 28 days after the first dose of vaccination (group A), before and 14 days after the second dose of vaccination, 28 days and the 6 months after full vaccination, the levels of neutralizing antibody are detected.

10.5.2.2 Assessment

The level of neutralizing antibody 28 days after full vaccination is assessed as primary endpoint in immunogenicity. The difference of antibody level between the groups and the change of antibody level at each time point before and after vaccination are compared.

10.5.3 Detection of neutralizing antibodies against recombinant replication deficient human adenovirus type 5

10.5.3.1 Detection time point

Before the first dose of vaccination in each group, 28 days after the first dose of vaccination (group A), before and 14 days after the second dose of vaccination, 28 days and the 6 months after full vaccination, the level of neutralizing antibodies against recombinant replication deficient human adenovirus type 5 are detected.

10.5.3.2 Assessment

The levels and GMI of neutralizing antibody against human adenovirus type 5 are compared before and after vaccination and in different groups. The correlation of SARS-CoV-2 neutralizing antibody level and IgG level to neutralizing antibodies against recombinant replication deficient human adenovirus type 5 at baseline are explored.

10.5.4 Detection of IFN - γ secretion by specific T cells (ELISPOT)

10.5.4.1 Detection time point

Before and 14 days after the first dose of vaccination in each group (group A not included), before and 14 days after the second dose of vaccination, the levels of IFN - γ stimulating by S pooled peptide are detected.

10.5.4.2 Assessment

The differences of IFN - γ positive cells ratios between groups, and proportion of IFN - γ positive at different time points before and after vaccination are compared.

10.5.5 Detection of specific CD4 + T cells and CD8 + T cells

10.5.5.1 Detection time point

Before and 14 days after the first dose of vaccination in each group (group A not included), before and 14 days after the second dose of vaccination, The specific CD4 + T cells and CD8 + T cells reactions are detected by flow cytometry.

10.5.5.2 Assessment

The difference of T cell reaction level between the groups and at each time point before and after vaccination are compared.

10.5.6 IgA test (group A and G not included)

10.5.6.1 Detection time point

Before and 14 days after the first dose of vaccination in each group, 14 days and 28 days after the second dose of vaccination, the levels of IgA antibody measured by ELISA are detected.

10.5.6.2 Assessment

Difference of IgA antibody level between groups and time point before and after vaccination are compared.

10.5.7 Monitoring and laboratory diagnosis of COVID-19 infection during the study

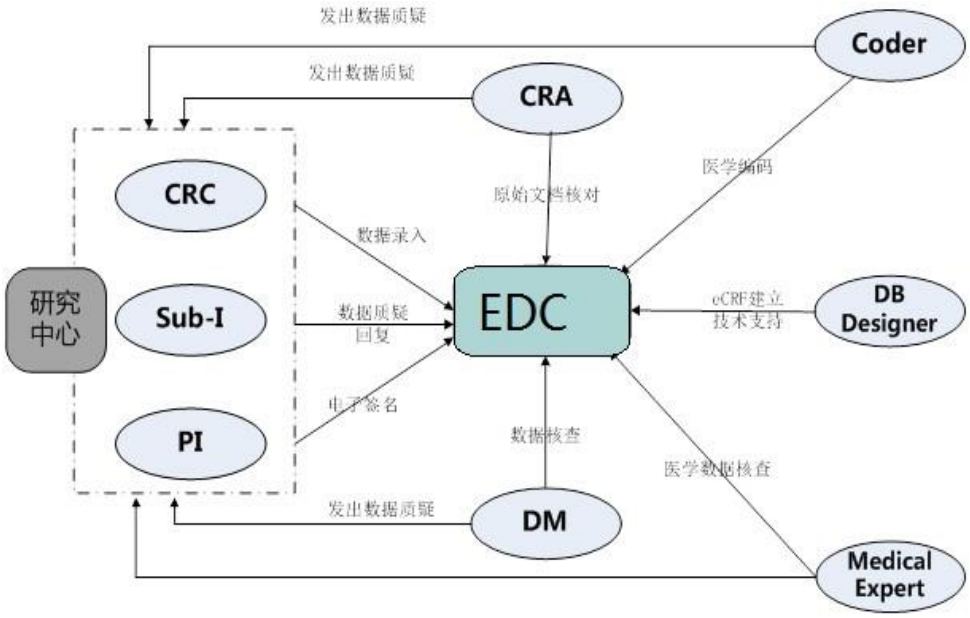
During the observation period of the clinical study, subjects with persistent fever, cough etc respiratory symptoms should immediately go to the designated hospital (Zhongnan Hospital of Wuhan University) and inform the investigators. Throat swabs / sputum should be collected and CT and other imaging examinations should be performed to determine whether the disease was caused by novel coronavirus infection. In case of novel coronavirus infection during the clinical study, case investigation should be carried out, and especially for those severe or dead cases special attention should be paid, mainly to analyze whether there is ADE phenomenon.

2019 novel coronavirus nucleic acids is detected from throat swabs and sputum specimens; additionally multi-pathogen detection also be carried out for differential diagnosis.

10.6 Data management

In this study, EDC system is used to collect and manage the research data, and the system retains the complete modification track to ensure the authenticity, integrity and accuracy of clinical study data; the data management process needs to comply with GCP specification to ensure the traceability of clinical study data.

10.6.1 Flow chart of data management



10.6.2 EDC system data acquisition roles and responsibilities

Role of EDC	Abbreviation	Responsibility
Clinical Data Coordinator	CRC	1、Data input ； 2、Q & A；
Assistant investigator	Sub-I	1、Data input ； 2、Q & A；
Principle investigator	PI	1、Data input ； 2、Q & A； 3、Electronic signature confirmation；
Clinical Research Associate	CRA	1、Source document verification (verify) 2、Raise query； 3、Close query：
Project manager	PM	1、Read ；
Data management	DM	1、Raise query；； 2、Close query： 3、Database Lock / Open； 4、Data Lock；
Medical coding	coder	1、Coding； 2、Raise query.

10.6.3 Design and establishment of database

The project database (ECRF) is established by the database designer, and the establishment of the database adopts the CDISC standard.

After the database is established and tested, it can be used online. Each one who has authorized role, e.g. PI, sub-I, CRC, PM, CRA, DM personnel etc can use EDC system after training.

The data manager writes the data management plan (DMP), which should be finalized before the first subject screening.

10.6.4 Data entry

The investigator or the person authorized by the investigator completes the online data entry in time after the visit. Investigator needs to approve the data on ECRF to confirm the records is true electronically. After the completion of data entry, any data changes need to be explained (comments) and will be automatically recorded in the system.

10.6.5 Monitoring of data records

The CRA shall monitor the data records entered into EDC to ensure that all the data entered are consistent with the original documents, regularly or irregularly. If there is any inconsistency, the monitor needs to send a query to the investigator at the corresponding place in the EDC system. The investigator needs to verify the original data and update the entered content until the data entry is completed in EDC system. Before locking the database, the monitor should carefully verify the original data of the subjects and the signature of the responsible investigator.

10.6.6 Data verification

The data manager checks the study data according to the data verification plan (DVP).

When the data is entered into EDC system, if there is any illogical mistake, the system will automatically check and ask questions (query); these queries need to be reviewed and answered by investigator or authorized personnel. When the updated data makes the logical verification not tenable, query will be automatically closed; When the query is automatically closed, DM can verify it; if the problem is not solved, DM can add queries manually and continue to communicate with the research center until the problem is solved.

In addition to the automatic verification of the system, when query id discovered by data manager manually, and it needs to clarify / verify / confirm the questions with the investigator, data manager can add Query manually into the EDC system.

Before locking the database, the data manager should make sure that all queries are cleaned up, and the investigator completes the electronic signature on the EDC system to ensure the integrity and accuracy of subject data.

10.6.7 Medical coding

Medical coders carry out medical coding. Medical history and adverse events will be coded according to MedDRA (23.1) dictionary.

In the process of coding, DM can query investigator online and in real time for any medical terms that cannot be coded due to improper, inaccurate and fuzzy provision.

Before locking the database, the medical code needs to be reviewed and approved.

10.6.8 Database locking

Complete the list of locking data. According to the procedure of database locking, the data manager, statistical analyst, clinical monitor representative and investigator representative sign and approve the database lock file in written. The data manager will export it to the database in the specified format and submit it to the statistical analysis personnel. After the data is locked, if there is definite evidence that it is necessary to unlock, investigator and relevant personnel need to sign the unlocking document.

10.6.9 External data management

Immunogenicity data is managed as external data. The data transmission requirements are detailed in 《external data transmission protocol》. Data management is used for external data audit and consistency check etc.

10.6.10 eCRF Archive

At the end of the study, the eCRF of each subject will be exported to PDF for electronic filing, and the CD will be stored in the clinical study center of Zhongnan Hospital of Wuhan University for 5 years after the study completion.

10.7 Statistical planning and analysis

10.7.1 Statistical plan

The statistical analysis of this study is divided into two parts: the first analysis and the final analysis.

10.7.1.1 First analysis

The last subject completes the 28th day visit of the last dose, data manager will sort out the research data, and make statistical analysis and summary of safety and immunogenicity.

10.7.1.2 Final analysis

At the end of the 6 months of the last visit of the last subject, the study data will be collected

for final analysis.

10.7.2 Statistical analysis plan

The sponsor entrusts the statistician and DSMB independent statistical team to undertake the task of statistical analysis and participate in the whole process from experimental design, implementation to analysis and summary. After the formulation of the experimental scheme is completed and approved by the ethics committee, the sponsor is responsible for coordinating the statistician and DSMB independent statistical team to establish the database and formulate the statistical plan, to determine the analysis data set and statistical methods (See 《Statistical analysis plan》 for details）

10.7.3 Selection of analysis data set

Safety evaluation data set (SS)

The safety evaluation should be carried out for all the subjects who received vaccination randomly grouped (except group A) and had post vaccination safety evaluation. Data violating the scheme should not be eliminated.

Immunogenicity evaluation data set

Full analysis data set (FAS): FAS is an ideal population determined according to the principle of ITT (intention to treat). All subjects who meet the inclusion / exclusion criteria, participate in randomization (except group A), receive immunization, and have at least one blood test result after immunization are included in the FAS set.

Per Protocol Set (PPS): it is a subset of FAS. The subjects in the data set are more compliant with the protocol, have no major violation of the protocol, meet the inclusion / exclusion criteria, and complete the vaccination within the vaccination time window according to the protocol requirements, and the subjects with blood collection at days of 0, 14, 28 and 6 months are included in the PPS set. This method was not included in the case of violation of the study plan, such as those with poor compliance or missing visits, and 2019 cases of new coronavirus infection.

In this study, FAS was used as the main analysis set. The first analysis of immunogenicity was only carried out in FAS, but PPS was also analyzed in the final analysis. If there was any inconsistency between PPS and FAS, it should be discussed in the report.

10.7.4 Data statistics method

The analysis of this study is based on description without statistical hypothesis. Except for missing of the start and end dates of adverse events (AE), while other data analysis did not fill in the missing data. During the statistical analysis, the number of completed cases and the shedding of cases were first checked; then the demographic and baseline characteristics of each group were analyzed to investigate the comparability between groups; the evaluation of vaccine effect included descriptive statistics of evaluation indexes and comparison of effects between groups; the safety evaluation included statistics of clinical adverse reactions / events.

Exclusion criteria: those who did not meet the inclusion criteria; those who failed to follow up the data and information after vaccination; those who lacked serious information and data after randomization; those who met the withdrawal criteria but did not withdraw; those who received wrong vaccination or incorrect doses.

In this study, the safety analysis was mainly descriptive analysis of the incidence rates of adverse reactions / adverse events. Chi square test was used for comparison between groups, and Fisher exact probability method was used if necessary.

The analysis of immunogenicity index of antibody level needs logarithmic transformation, which should be expressed by GMT, standard deviation, median, maximum and minimum value and 95% confidence interval. The comparison of classification indexes between groups, such as antibody positive conversion rate, should be performed by chi square test, and Fisher exact probability method if necessary.

SAS 9.4 or above version statistical analysis software was used for statistical analysis of all statistical calculations. Two sided test was used for statistical analysis. The test statistics and corresponding p value were given, Fisher exact probability method was used to calculate the p value directly, and $P \leq 0.05$ was taken as the standard with statistical significance (see

《Statistical analysis plan》 for details).

11. Monitoring of clinical study

11.1 Responsibilities

The quality assurance system shall be implemented and maintained by the sponsor to ensure that the test is carried out in accordance with the provisions, and the study data collection, recording and reporting shall meet the requirements of GCP and study plan. Researchers and monitors should have a comprehensive grasp of the clinical study protocol and all relevant procedures, including the information of the study vaccine, the procedures for obtaining informed consent, the reporting procedures of adverse events (including serious adverse events), and the procedures for completing eCRF.

The principle researcher should clearly authorize and manage all the researchers participating in the clinical study, and formulate SOP for each research post.

Researchers should keep their personal data confidentially. eCRF or other documents provided to the sponsor shall be identified only by codes or random numbers. The identification code (including full name, age and address) of the subject is saved by the researcher in the researcher document. According to the GCP principle, the original data of each subject is allowed to be monitored, checked and reviewed by the drug administration department.

Shanghai STEM Pharmaceutical Development Co., Ltd. (CRO) is responsible for clinical monitoring of sites and researchers, including quality control, medical monitoring, pharmacovigilance, statistical analysis and summary of clinical studys. The auditor should follow up the site according to a certain time schedule. During the audit, it is necessary to check whether the original data are consistent with the information on eCRF, that is, its accuracy and completion. If it is found that the eCRF is inconsistent with the original data, it is necessary to urge the researchers to modify or complete it as soon as possible. The auditors will evaluate the process of informed consent, the transportation and storage of the study vaccine, the study documents and the progress of the study. The auditor should check the compliance of the researcher to the protocol (or protocol amendment), observe the study procedure and discuss

some problems with the researcher. There should be monitoring records to record the on-site monitoring. After the study, the auditor shall provide the sponsor with a copy of the audit record.

The clinical study data safety monitoring committee carries out the monitoring of the clinical study according to the constitution, and independently analyzes the safety data of the subjects after vaccination. If the clinical study data safety monitoring committee finds that the risk of the subjects increases during the research process, it needs to immediately inform the principle researcher and the sponsor to suspend or terminate the clinical study.

Institute of Biotechnology, Academy of Military Medical Sciences, and Zhongnan Hospital of Wuhan University are responsible for the overall design, organization arrangement, formulation of relevant technical schemes, and writing research summary.

Pengcheng (Beijing) Pharmaceutical Technology Co., Ltd. was responsible for the recruitment of subjects. Beijing Sterig Medical Technology Co., Ltd. (SMO) assisted the Zhongnan Hospital of Wuhan University in non-medical assessment work such as subject screening, registration, informed consent, physical examination, discharge determination, sample collection, observation, safety follow-up assistance, and assisted the researchers in site killing and medical waste treatment.

Zhongnan Hospital of Wuhan University and I Institute of Biotechnology, Academy of Military Medical Sciences are responsible for immunogenicity test and submitting test report.

11.2 Quality control of vaccine

The study vaccine should be managed by authorized person and supervised by the monitor. The number of vaccines received, the number of vaccinated subjects, the remaining number and the loss number should be recorded in detail, which should be recorded in the work log.

The sponsor is responsible for the delivery of the study vaccine. When investigator finds that the vaccine package is damaged, the vaccine is deteriorated or there are lumpy substances that cannot be shaken away, do not use it and return it to the sponsor. If the cold chain system is damaged or the vaccine is frozen during transportation and storage, the vaccine can not be used. It should be stored separately and clearly marked, managed by assigned person and returned to

the sponsor. The investigator must sign the corresponding record to confirm the receipt of all vaccines. The receipt record should briefly state the information of the vaccines received, including the number of vaccines, whether the packaging is in good condition, and whether the cold chain system indication is normal. At the end of the study, the investigator should calculate the quantity of all the remaining vaccines, and after use, the inner packaging (pre filled syringe) should be destroyed as medical waste at the study site. After use, the total number of outer packaging and remaining vaccines should be consistent with the number of vaccines received by the investigator. All outer packaging and remaining vaccines should be returned to the sponsor. The investigator should fill in the corresponding records and confirm the acceptance of the sponsor.

When returning the vaccine, the investigator shall return the vaccine handover form to the sponsor. The investigator is responsible for explaining any doubt in the number of vaccines.

11.3 Quality control of documents

11.3.1 Source Document

Source data includes the demographic data, medical history inquiry results, physical examination results, laboratory test results, vaccination records, blood collection records, concomitant medication, adverse events / reactions and their treatment and outcome of the subjects. All the information should be recorded in the source document, and the investigator should properly keep it in a special room. The source document will be archived in Zhongnan Hospital of Wuhan University, which is the basis for the subjects to participate in clinical study and the accuracy and integrity of the data.

Investigator should carefully, accurately and timely fill in the vaccination and visit records and other source records. All the source data collected should be recorded in the vaccination and visit records on the day it when is collected, and the writing and modification should be standardized. The source record shall include the following basic data:

study name, screening number and study number

- Demographics

- Inclusion / exclusion criteria

- Results of physical examination

- Laboratory test results (excluding immunological test)

- Vaccination records

- The date of follow-up and the date of discontinuation of the study

- Adverse events / reactions and the treatment and outcome

- Blood collection records

- Concomitant medication, medical treatment and other vaccinations

11.3.2 Electronic case report form (eCRF)

Only investigator and approved staff are allowed to visit eCRF during the study. For the subjects who are discontinued from the study earlier, the reason for discontinuation should be recorded on eCRF.

eCRF should reflect the situation of subjects in each stage of the experiment. The name of the subject cannot be filled in eCRF, and the appropriate code and the subject's initials must be used.

All the data in eCRF are from the source document and are consistent with the source document. All data recorded in eCRF should be recorded in the source document.

The clinical study monitor entrusted by the sponsor has the right to view eCRF, informed consent and all source document at any time.

The sponsor, investigator and other relevant personnel shall provide written documents for clinical study communication, meeting, any modification of protocol and SOP, and all documents mutually agreed by both parties shall be in duplicate and kept for record.

11.3.3 Documentation

The data of clinical studys should be kept according to the requirements of GCP. The

investigator should keep the study data for at least 5 years after the end of clinical studies. The data of clinical studies that should be kept by the sponsor will be kept permanently.

11.4 Quality control of biological samples

The collection, treatment, storage and transportation of biological samples are in strict accordance with the operation manual.

11.5 Ownership and publication

All research information provided by the sponsor and all data / information generated in the research center (except medical records of subjects) are owned by the sponsor. If the terms of publication involved in the written contract of this study contradict to this statement, the terms of the contract shall prevail.

Before publishing research results in the form of contribution, speech, teaching or other forms (collectively referred to as "publication"), the investigator must submit a copy of the planned publication content to the sponsor and obtain written approval before publishing. The contents of the plan should not include the confidential information of the sponsor and the personal information (such as name or acronym) of the subjects that are not the results of the study.

11.6 Confidentiality

The sponsor, the investigator, the representative of the Ethics Committee (IEC) or the fully authorized management organization shall have the right to obtain the data related to the clinical study, but the relevant contents can not be used in any other clinical study nor be disclosed to any other person or entity. The investigator must sign a confidentiality agreement to confirm that he knows and agrees to be responsible for the confidentiality of the information in this study.

Investigators and other researchers shall keep confidential for all information provided by the sponsor and all data / information generated in the research center (except medical records of subjects). This information and data cannot be used for any purpose other than research. This restriction does not apply to: (1) the research information is not disclosed due to the violation of regulations by investigators and researchers; (2) the research information is only disclosed

to IRB / IEC for the purpose of research evaluation; (3) the research information is disclosed in order to provide appropriate medical assistance to subjects; (4) the research results authorized by the sponsor are published. If the confidentiality clause involved in the written contract of this study contradicts to this statement, the contract clause shall prevail.

12. Time table

It will take about 13 months for this study from the preparation before the study to the final completion of the final report (only for reference)

Implementation process	Estimated duration
1.Preparation before clinical study	30 days
2. EC review and approval	5 days
3.The first subject recruitment	2 months
4.The last subject completed visit 2	
5. First analysis	10 days
6.First analysis report	
7. The last subject completed the visit 7	8 months
8.Final analysis	1 month
9.Clinical study report	14 days

13. Ethical approval

13.1 Ethical review and approval

The principle investigator submits the clinical study protocol and all necessary additional documents to the Ethics Committee for initial review

-Clinical study protocol (indicate version number / date)

-Informed consent form (with version number / date)

-Materials for subject recruitment (indicate version number / date)

-eCRF sample (indicate version number / date)

-Diary card (indicate version number / date)

-Contact card (indicate version number / date)

-Vaccination and visit records (indicate version number / date)

-Investigator's Brochure

-Curriculum vitae of principle investigator

-Approval documents of military special drugs

-Research vaccine test report or batch issuing document

-After the above research documents are approved by the ethics committee, a written approval certificate shall be issued to the investigator. The investigator shall provide a copy of the written approval certificate to the sponsor.

13.2 Follow up review

Whether the method of selecting subjects and providing relevant information to subjects are complete and easy to understand; whether the method of obtaining informed consent is appropriate; whether the SAE report is in time; if the subjects have SAE related to the experimental vaccination, whether they can get timely medical treatment.

In the whole process of the study, the ethics committee should supervise whether the risk benefit ratio of the study is increased and whether the rights and interests of the subjects are effectively protected.

13.3 Potential hazards and risk minimization

13.3.1 Benefits and risks

Subjects in this clinical study are not required to pay for the vaccination of the research vaccine. Because of participating in this experiment, the subjects can get reasonable transportation expenses, work delay expenses, blood collection compensation and nutrition expenses. The

novel coronavirus vaccine (adenovirus vector) will be inoculated in the clinical study. The novel coronavirus novel coronavirus (COVID-19) threat may be prevented from being infected by 2019 novel coronavirus within a certain period of time. At the same time, vaccination may cause some adverse reactions, including fever, injection site pain, swelling and so on. Usually, the adverse reactions will be relieved within 3-5 days. In the clinical research of adenovirus vaccine abroad, it has been reported that the adenovirus vector may cause the prolongation of coagulation time for a period of time, but it generally does not affect the safety of life. Adenovirus vector vaccines have been approved for marketing abroad. The recombinant Ebola virus disease vaccine based on the same adenovirus vector platform has been approved in China, showing good safety in practical use. In addition, in the clinical study of VSV vector vaccine in Canada, it was found that vaccination may cause joint pain, which should be observed in the study.

During the safety observation period, if adverse events or serious adverse events occur, the subjects will receive timely treatment. The sponsor also bought commercial insurance for the subjects. The commercial insurance company will bear the reasonable expenses for diagnosis and treatment, work delay and transportation, and the reasonable compensation beyond the scope of insurance will be borne by the sponsor.

Currently no novel coronavirus vaccine is available in China. If the subjects are unwilling to be vaccinated with the study vaccine, no other coronavirus vaccine can be replaced.

13.3.2 Vaccination

To avoid adverse events caused by improper inoculation or inoculation errors, we should purchase qualified inoculation consumables and carry out aseptic inoculation in strict accordance with the standard methods.

In case of grade 3 or above adverse reactions, or SAE related to or possibly related to the study vaccination during the safety observation period, the subjects should be able to receive timely medical treatment, and the "green channel for medical treatment" should be started immediately for emergency treatment if necessary.

13.3.3 Blood specimen collection

After the qualification examination of the principle investigator, experienced nursing staff will be employed to collect venous blood samples according to the specified procedures after training, so as to minimize the pain or risk (including pain and infection of the puncture site with little probability) of the subjects.